METHODS AND COMPOSITIONS FOR TREATMENT OF RETINAL DEGENERATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/711,665, filed October 9, 2012, the disclosure of which is hereby incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERAL SUPPORT

[0002] Not applicable.

FIELD

[0003] The present application is in the field of cell therapies for retinal degeneration as occurs, for example, in retinitis pigmentosa and in age-related macular degeneration (AMD).

BACKGROUND

[0004] Retinal degeneration, resulting, for example, from choroidal neovascularization ("wet AMD") or from buildup of cellular debris between the retina and the choroid ("dry AMD"), is one of the major causes of blindness in the world today. Cai et al. (2012) *Front Biosci.* **17**:1976-95. Similarly, degeneration and death of photoreceptor cells (rods and cones), as occurs in Retinitis pigmentosa, can also lead to deterioration and/or loss of vision. Accordingly, treatments that block and/or reverse retinal degeneration, in particular treatments that restore photoreceptor function, are needed.

SUMMARY

[0005] Disclosed herein are methods and compositions for treating retinal degeneration, using cells descended from marrow adherent stem cells (MASCs) that have been engineered to express an exogenous Notch intracellular domain. Such cells are denoted SB623 cells for the purposes of the present disclosure.

[0006] In one example, disclosed herein are methods of treating retinal degeneration by administering cells to the eye of a subject in need thereof, wherein said cells are descendants of marrow adherent stem cells that have been

engineered to express an exogenous Notch intracellular domain. The present invention disclosed herein refers to the use of cells in the manufacture of a medicament for treating retinal degeneration in a subject in need thereof, wherein said cells are descendants of marrow adherent stem cells that have been engineered to express an exogenous Notch intracellular domain.

[0007] In another example, disclosed herein are methods of increasing photoreceptor activity in the eye of a subject, the methods comprising administering cells to the eye of the subject such that photoreceptor activity is increased, wherein said cells are descendants of marrow adherent stem cells that have been engineered to express an exogenous Notch intracellular domain.

[0008] In another example, disclosed herein are methods of enhancing photoreceptor function in the eye of a subject, the methods comprising administering cells to the eye of the subject such that photoreceptor function is enhanced, wherein said cells are descendants of marrow adherent stem cells that have been engineered to express an exogenous Notch intracellular domain.

[0009] In another example, disclosed herein are methods of enhancing transmission of visual signals from the retina to the visual cortex of the brain, the methods comprising administering cells to the eye of the subject such that transmission of visual signals from the retina to the visual cortex of the brain is enhanced, wherein said cells are descendants of marrow adherent stem cells that have been engineered to express an exogenous Notch intracellular domain.

[0010] In any of the methods described herein, the cells can be administered by any delivery method, including direct injection, topical administration and the like. In certain examples, the cells are administered as a composition (or formulation) comprising the cells, for example in combination with one or more pharmaceutical carriers. In addition, the methods can involve repeated administration of the cells, in the same or different formulations.

[0011] Accordingly, the present disclosure describes, *inter alia*, the following examples:

1. A method for treating retinal degeneration in a subject in need thereof, the method comprising administering cells to the subject, wherein said cells are descendants of marrow adherent stem cells that have been engineered to express an exogenous Notch intracellular domain.

2. The method of example 1, wherein said cells are transplanted into the eye of the subject.

3. The method of either of examples 1 or 2, wherein the transplantation is intravitreal.

4. The method of either of example 1 or 2, wherein the transplantation is subretinal.

5. The method of any of examples 1-4, wherein the retinal degeneration occurs in retinitis pigmentosa.

6. The method of any of examples 1-4, wherein the retinal degeneration occurs in age-related macular degeneration (AMD).

[0012] These and other aspects will be readily apparent to the skilled artisan in light of disclosure as a whole.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **Figure 1** shows representative electroretinogram (ERG) traces from the eyes of RCS rats at 4 weeks after birth (prior to treatment, top set of panels), 8 weeks after birth (4 weeks after treatment, second set of panels from top) and 12 weeks after birth (8 weeks after treatment, third set of panels from top). Rats were treated at 4 weeks after birth by intravitreal injection of either 1.5 x 10^5 SB623 cells (right panels) or PBS (left panels). The bottom set of panels shows photoreceptor activity as assayed by azide responses at 12 weeks after birth (8 weeks after treatment) for rats that were treated at 4 weeks after birth by intravitreal injection of either 1.5 x 10^5 SB623 cells (right panel) or PBS (left panel).

[0014] **Figure 2, panels A and B,** shows a set of graphs depicting relative amplitudes of a-waves (Figure 2A) and b-waves (Figure 2B) from electroretinograms of RCS rats taken at 4, 5, 6, 8 and 12 weeks after birth (*i.e.*, pre-treatment and at 1, 2, 4 and 8 weeks after treatment). For each set of bars, the left-most bar represents the value for naïve (*i.e.* untreated) animals. Proceeding rightward, the remaining bars represent values for animals treated by intravitreal injection of vehicle, 0.375 x 10^5 SB623 cells, 0.75 x 10^5 SB623 cells and 1.5 x 10^5 SB623 cells. Numbers in parentheses indicate the number of eyes analyzed. Pretreatment values were set as 100%.

[0015] **Figure 3** is a graph showing amplitudes (in microvolts) of the azide response in eyes of RCS rats at 12 weeks after birth (8 weeks after treatment). Animals were untreated ("Naïve") or subjected to intravitreal injection, at 4 weeks of age, with PBS ("Vehicle"), 0.375 x 10^5 SB623 cells, 0.75 x 10^5 SB623 cells, or 1.5 x 10^5 SB623 cells. Numbers in parentheses indicate the number of eyes analyzed.

[0016] **Figure 4, panels A and B,** shows hematoxylin and eosin (H&E)-stained sections of RCS rat retina at 9 weeks after treatment. Figure 4B shows a section from an eye of a rat treated, at 4 weeks after birth, by intravitreal injection of 1.5 x 10^5 SB623 cells. Figure 4A shows a section from an eye of a control rat into which PBS was injected at 4 weeks after birth. A well-developed outer nuclear layer (indicated "ONL" in the figure) is present in the SB623-treated eyes, but absent in vehicle-treated eyes.

[0017] **Figure 5, panels A to D,** shows sections of retinas from RCS rats nine weeks after intravitreal injection of 1.5 x 10^5 SB623 cells (13 weeks postnatal).

Figures 5A and 5C show H&E-stained sections; Figures 5B and 5D show sections stained with anti-human mitochondria antibody (green) and counterstained with the nucleus-specific dye DAPI (blue). The two upper panels show a section containing a clump of SB623 cells in the vitreous body. The two lower panels show a section of retina in which a SB623 cell can be seen on the inner limiting membrane of the retina.

[0018] **Figure 6** shows representative electroretinogram (ERG) traces from the eyes of RCS rats at 4 weeks after birth (prior to treatment, top set of panels), 8 weeks after birth (4 weeks after treatment, second set of panels from top) and 28 weeks after birth (24 weeks after treatment, third set of panels from top). Rats were treated at 4 weeks after birth by subretinal injection of either 1.5×10^5 SB623 cells (right panels) or PBS (left panels). The bottom set of panels shows photoreceptor activity as measured by azide responses at 28 weeks after birth (24 weeks after treatment) for rats that were treated at 4 weeks after birth by subretinal injection of either 1.5×10^5 SB623 cells (right panel) or PBS (left panel).

[0019] **Figure 7, panels A and B,** shows a set of graphs depicting relative amplitudes of a-waves (Figure 7A) and b-waves (Figure 7B) from electroretinograms of RCS rats taken pre-treatment and at 4, 8, 12, 16, 20 and 24 weeks after treatment. For each set of bars, the left-most bar represents the value for naïve (*i.e.* untreated) animals; the middle bar represents values for animals treated by subretinal injection of vehicle; and the right-most bar represents values for animals treated by subretinal injection of 1.5×10^5 SB623 cells. Numbers in parentheses indicate the number of eyes analyzed. Pretreatment amplitude was set as 100%.

[0020] **Figure 8** is a graph showing amplitudes (in microvolts) of the azide response in eyes of RCS rats at 4, 8, 12, 16, 20 and 24 weeks after treatment. For each set of three bars, the left-most bar represents the value for naïve (*i.e.* untreated) animals; the middle bar represents values for animals treated by subretinal injection of vehicle; and the right-most bar represents values for animals treated by subretinal injection of 1.5×10^5 SB623 cells. Numbers in parentheses indicate the number of eyes analyzed.

[0021] **Figure 9** shows traces of visually evoked potential (VEP), taken 26 weeks after subretinal transplantation, from naïve, vehicle-treated and SB623 cell-treated RCS rats.

[0022] **Figure 10, panels A and B,** shows hematoxylin and eosin (H&E)-stained sections of RCS rat retina at 27 weeks after treatment. Figure 10B shows a

section from an eye of a rat treated, at 4 weeks after birth, by subretinal injection of 1.5×10^5 SB623 cells. Figure 10A shows a section from an eye of a control rat into which PBS was injected at 4 weeks after birth. A well-developed outer nuclear layer (indicated "ONL" in the figure) is present in the SB623-treated eyes, but absent in vehicle-treated eyes.

[0023] **Figure 11, panels A and B,** shows sections of retina from RCS rats 27 weeks after subretinal injection of 1.5×10^5 SB623 cells (31 weeks postnatal). Figure 11A shows a H&E-stained section; Figure 11B shows a section stained with anti-human mitochondria antibody (green) and counterstained with the nucleus-specific dye DAPI (blue). Transplanted SB623 cells are visible in the Figure 11A (arrowheads).

DETAILED DESCRIPTION

[0024] Disclosed herein are methods and compositions for the treatment of retinal degeneration and retinal degenerative conditions. In particular, transplantation of SB623 cells (cells obtained by transfecting mesenchymal stem cells with sequences encoding a Notch intracellular domain) into the eyes of subjects undergoing retinal degeneration (or suffering from a retinal degenerative condition) prevents retinal degeneration and results in long-term rescue of retinal function.

[0025] Practice of the present disclosure employs, unless otherwise indicated, standard methods and conventional techniques in the fields of cell biology, toxicology, molecular biology, biochemistry, cell culture, immunology, oncology, recombinant DNA and related fields as are within the skill of the art. Such techniques are described in the literature and thereby available to those of skill in the art. See, for example, Alberts, B. *et al.*, "Molecular Biology of the Cell," 5th edition, Garland Science, New York, NY, 2008; Voet, D. *et al.* "Fundamentals of Biochemistry: Life at the Molecular Level," 3rd edition, John Wiley & Sons, Hoboken, NJ, 2008; Sambrook, J. *et al.*, "Molecular Cloning: A Laboratory Manual," 3rd edition, Cold Spring Harbor Laboratory Press, 2001; Ausubel, F. *et al.*, "Current Protocols in Molecular Biology," John Wiley & Sons, New York, 1987 and periodic updates; Freshney, R.I., "Culture of Animal Cells: A Manual of Basic Technique," 4th edition, John Wiley & Sons, Somerset, NJ, 2000; and the series "Methods in Enzymology," Academic Press, San Diego, CA.

Retinal degeneration

[0026] Two of the most commonly-occurring retinal degenerative conditions are retinitis pigmentosa (RP) and age-related macular degeneration (AMD). Retinitis pigmentosa results from degeneration of the photoreceptor cells of the retina, also known as rods and cones. The macula is the name given to the central portion of the retina and is responsible for central, as opposed to peripheral, vision. There are two forms of AMD. The more common form, dry AMD, is caused by the buildup of cellular debris (drusen) between the retina and the choroid (the layer of the eye beneath the retina), leading to atrophy of photoreceptor cells. The other form of AMD, wet AMD, results from abnormal growth of blood vessels in the choroid. These vessels may leak, resulting in damage to the choroid and the retina. Other terms for AMD include choroidal neovascularization, subretinal neovascularization, exudative form and disciform degeneration.

[0027] Other types of retinal degenerative conditions include Usher syndrome (an inherited condition characterized by hearing loss and progressive loss of vision from RP), Stargardt's disease (inherited juvenile macular degeneration), Leber Congenital Amaurosis (an inherited disease characterized by loss of vision at birth), choroideremia (an inherited condition causing progressive vision loss due to degeneration of the choroid and retina), Bardet-Biedl syndrome (a complex of disorders that includes retinal degeneration and can also include polydactyly and renal disease), and Refsum disease (a disorder caused by inability to metabolize phytanic acid which is characterized by, *inter alia*, RP). *See, e.g.,* Goodwin (2008) *Curr Opin Ophthalmol* **19(3):**255-62; Bonnet *et al.* (2012) *Curr Opin Neurol.* **25(1):**42-9; Coussa *et al.* (2012) *Ophthalmic Genet.* **33(2):**57-65.

[0028] Other, rarer retinal degenerative conditions that can be treated using the methods and compositions described herein include Best's disease, cone-rod retinal dystrophy, gyrate atrophy, Oguchi disease, juvenile retinoschisis, Bassen-Kornzweig disease (abetalipoproteinemia), blue cone monochromatism disease, dominant drusen, Goldman-Favre vitreoretinal dystrophy (enhanced S-cone syndrome), Kearns-Sayre syndrome, Laurence-Moon syndrome, peripapillary choroidal dystrophy, pigment pattern dystrophy, (including Butterfly-shaped pigment dystrophy of the fovea, North Carolina macular dystrophy, macro-reticular dystrophy, spider dystrophy and Sjogren reticular pigment epithelium dystrophy), Sorsby

macular dystrophy, Stickler's syndrome and Wagner's syndrome (vitreoretinal dystrophy).

SB623 cells

[0029] The present disclosure provides methods for treating retinal degeneration by transplanting SB623 cells into the eye of a subject in need thereof, namely a subject in which retinal degeneration is occurring. SB623 cells are obtained from marrow adherent stromal cells (MASCs), also known as mesenchymal stem cells (MSCs), by expressing the intracellular domain of the Notch protein in the MASCs. MASCs are obtained by selecting adherent cells from bone marrow.

[0030] In one embodiment, a culture of MASCs is contacted with a polynucleotide comprising sequences encoding a NICD (*e.g.*, by transfection), followed by enrichment of transfected cells by drug selection and further culture. See, for example, U.S. Patent No. 7,682,825 (issued March 23, 2010); U.S. Patent Application Publication No. 2010/0266554 (Oct. 21, 2010); and WO 2009/023251 (Feb. 19, 2009); all of which disclosures are incorporated by reference, in their entireties, for the purposes of describing isolation of mesenchymal stem cells and conversion of mesenchymal stem cells to SB623 cells (denoted "neural precursor cells" and "neural regenerating cells" in those documents). *See* also Example 1, *infra*.

[0031] In these methods, any polynucleotide encoding a Notch intracellular domain (*e.g.*, vector) can be used, and any method for the selection and enrichment of transfected cells can be used. For example, in certain embodiments, MASCs are transfected with a vector containing sequences encoding a Notch intracellular domain and also containing sequences encoding a drug resistance marker (*e.g.* resistance to G418). In additional embodiments, two vectors, one containing sequences encoding a Notch intracellular domain and the other containing sequences encoding a drug resistance marker, are used for transfection of MASCs. In these embodiments, selection is achieved, after transfection of a cell culture with the vector or vectors, by adding a selective agent (*e.g.*, G418) to the cell culture in an amount sufficient to kill cells that do not comprise the vector but spare cells that do. Absence of selection entails removal of said selective agent or reduction of its concentration to a level that does not kill cells that do not comprise the vector. Following selection (*e.g.*, for seven days) the selective agent is removed and the cells are further cultured (*e.g.*, for two passages).

[0032] Preparation of SB623 cells thus involves transient expression of an exogenous Notch intracellular domain in a MSC. To this end, MSCs can be transfected with a vector comprising sequences encoding a Notch intracellular domain wherein said sequences do not encode a full-length Notch protein. All such sequences are well known and readily available to those of skill in the art. For example, Del Amo *et al.* (1993) *Genomics* **15:**259-264 present the complete amino acid sequences of the mouse Notch protein; while Mumm and Kopan (2000) *Devel. Biol.* **228:**151-165 provide the amino acid sequence, from mouse Notch protein, surrounding the so-called S3 cleavage site which releases the intracellular domain. Taken together, these references provide the skilled artisan with each and every peptide containing a Notch intracellular domain that is not the full-length Notch protein; thereby also providing the skilled artisan with every polynucleotide comprising sequences encoding a Notch intracellular domain that does not encode a full-length Notch protein. The foregoing documents (Del Amo and Mumm) are incorporated by reference in their entireties for the purpose of disclosing the amino acid sequence of the full-length Notch protein and the amino acid sequence of the Notch intracellular domain, respectively.

[0033] Similar information is available for Notch proteins and nucleic acids from additional species, including rat, *Xenopus*, *Drosophila* and human. *See*, for example, Weinmaster *et al.* (1991) *Development* **113:**199-205; Schroeter *et al.* (1998) *Nature* **393:**382-386; NCBI Reference Sequence No. NM_017167 (and references cited therein); SwissProt P46531 (and references cited therein); SwissProt Q01705 (and references cited therein); and GenBank CAB40733 (and references cited therein). The foregoing references are incorporated by reference in their entireties for the purposes of disclosing the amino acid sequence of the full-length Notch protein and the amino acid sequence of the Notch intracellular domain in a number of different species.

[0034] In additional embodiments, SB623 cells are prepared by introducing, into MSCs, a nucleic acid comprising sequences encoding a Notch intracellular domain such that the MSCs do not express exogenous Notch extracellular domain. Such can be accomplished, for example, by transfecting MSCs with a vector comprising sequences encoding a Notch intracellular domain wherein said sequences do not encode a full-length Notch protein.

[0035] Additional details on the preparation of SB623 cells, and methods for making cells with properties similar to those of SB623 cells which can be used in the

methods disclosed herein, are found in U.S. Patent No. 7,682,825; and U.S. Patent Application Publication Nos. 2010/0266554 and 2011/0229442; the disclosures of which are incorporated by reference herein for the purposes of providing additional details on the preparation of SB623 cells, and for providing methods for making cells with properties similar to those of SB623 cells. See also Dezawa *et al.* (2004) *J. Clin. Invest.* **113**:1701-1710.

Formulations, kits and routes of administration

[0036] Therapeutic compositions comprising SB623 cells as disclosed herein are also provided. Such compositions typically comprise the SB623 cells and a pharmaceutically acceptable carrier.

[0037] The therapeutic compositions disclosed herein are useful for, *inter alia*, reducing the progress of retinal degeneration, reversing retinal degeneration and/or restoring photoreceptor function. Accordingly, a "therapeutically effective amount" of a composition comprising SB623 cells can be any amount that prevents or reverses retinal degeneration and/or restores photoreceptor function. For example, dosage amounts can vary from about 100; 500; 1,000; 2,500; 5,000; 10, 000; 20,000; 50;000; 100,000; 500,000; 1,000,000; 5,000,000 to 10,000,000 cells or more (or any integral value therebetween); with a frequency of administration of, *e.g.*, once per day, twice per week, once per week, twice per month, once per month, depending upon, *e.g.*, body weight, route of administration, severity of disease, *etc.*

[0038] Various pharmaceutical compositions and techniques for their preparation and use are known to those of skill in the art in light of the present disclosure. For a detailed listing of suitable pharmacological compositions and techniques for their administration one may refer to texts such as Remington's Pharmaceutical Sciences, 17th ed. 1985; Brunton *et al.*, "Goodman and Gilman's The Pharmacological Basis of Therapeutics," McGraw-Hill, 2005; University of the Sciences in Philadelphia (eds.), "Remington: The Science and Practice of Pharmacy," Lippincott Williams & Wilkins, 2005; and University of the Sciences in Philadelphia (eds.), "Remington: The Principles of Pharmacy Practice," Lippincott Williams & Wilkins, 2008.

[0039] The cells described herein can be suspended in a physiologically compatible carrier for transplantation. As used herein, the term "physiologically compatible carrier" refers to a carrier that is compatible with the other ingredients of

the formulation and not deleterious to the recipient thereof. Those of skill in the art are familiar with physiologically compatible carriers. Examples of suitable carriers include cell culture medium (*e.g.*, Eagle's minimal essential medium), phosphate buffered saline, Hank's balanced salt solution+/-glucose (HBSS), and multiple electrolyte solutions such as Plasma-Lyte™ A (Baxter).

[0040] The volume of a SB623 cell suspension administered to a subject will vary depending on the site of transplantation, treatment goal and number of cells in solution. Typically the amount of cells administered will be a therapeutically effective amount. As used herein, a "therapeutically effective amount" or "effective amount" refers to the number of transplanted cells which are required to effect treatment of the particular disorder; *i.e.*, to produce a reduction in the amount and/or severity of the symptoms associated with that disorder. For example, in the case of treatment for AMD, transplantation of a therapeutically effective amount of SB623 cells typically results in prevention or reversal of retinal degeneration and/or restoration of photoreceptor function. Therapeutically effective amounts vary with the type and extent of retinal degeneration, and can also vary depending on the overall condition of the subject.

[0041] The disclosed therapeutic compositions can also include pharmaceutically acceptable materials, compositions or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, *i.e.*, carriers. These carriers can, for example, stabilize the SB623 cells and/or facilitate the survival of the SB623 cells in the body. Each carrier should be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical

formulations. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0042] Exemplary formulations include, but are not limited to, those suitable for parenteral administration, *e.g.*, intrapulmonary, intravenous, intra-arterial, intra-ocular, intra-cranial, sub-meningial, or subcutaneous administration, including formulations encapsulated in micelles, liposomes or drug-release capsules (active agents incorporated within a biocompatible coating designed for slow-release); ingestible formulations; formulations for topical use, such as eye drops, creams, ointments and gels; and other formulations such as inhalants, aerosols and sprays. The dosage of the compositions of the disclosure will vary according to the extent and severity of the need for treatment, the activity of the administered composition, the general health of the subject, and other considerations well known to the skilled artisan.

[0043] In additional embodiments, the compositions described herein are delivered locally. Localized delivery allows for the delivery of the composition non-systemically, thereby reducing the body burden of the composition as compared to systemic delivery. Such local delivery can be achieved, for example, through the use of various medically implanted devices including, but not limited to, stents and catheters, or can be achieved by inhalation, phlebotomy, injection or surgery. Methods for coating, implanting, embedding, and otherwise attaching desired agents to medical devices such as stents and catheters are established in the art and contemplated herein. Local delivery can also be achieved, for example, by intra-ocular injection or by application of eye drops.

[0044] Another aspect of the present disclosure relates to kits for carrying out the administration of SB623 cells to a subject. In one embodiment, a kit comprises a composition of SB623 cells, formulated as appropriate (*e.g.*, in a pharmaceutical carrier), in one or more separate pharmaceutical preparations.

Administration

[0045] For treatment of retinal degeneration (*e.g.*, AMD) with SB623 cells, any method known in the art for delivery of substances to the eye can be utilized. For the purposes of this disclosure, "transplantation" refers to the transfer of SB623 cells

to the eye of a subject, by any method. For example, direct injection into the eye can be used for delivery of a suspension of SB623 cells. In certain embodiments, a suspension of SB623 cells is injected into the vitreous humor. In other embodiments, subretinal injection is used. In additional embodiments, topical administration is used; for example, therapeutic compositions can be formulated in a solution to be used as eye drops. In still other embodiments, topical application of suspensions, gels and the like can utilized for administration of SB623 cells.

EXAMPLES

[0046] Proper function of photoreceptor cells involves continual synthesis and shedding of photoreceptor outer segments. Cells of the retinal pigmented epithelium (RPE cells) aid in this process by phagocytosing shed outer segments, and by recycling retinoids and membrane lipids.

[0047] The Royal College of Surgeons rat ("RCS rat") is an animal model of inherited retinal degeneration, in which retinal degeneration results from defective RPE cells that are unable to phagocytose photoreceptor outer segments. D'Cruz *et al.* (2000) *Human Molecular Genetics* **9(4):**645–651. Histologically, the retina of the RCS rat is characterized by abnormal accumulation of outer segment debris between the photoreceptor cell outer segment layer and the retinal pigmented epithelium. Accumulation occurs prior to, and concomitant with, the death of photoreceptor cells. RCS rats experience progressive postnatal loss of photoreceptor cells and attendant loss of vision.

[0048] Electroretinography is a process in which an electrode is placed on the cornea, the eye is stimulated by a flash of light, and the electrical activity of the photoreceptor cells is measured by the electrode. Odom JV, Leys M, Weinstein GW. Clinical visual electrophysiology. In: Tasman W, Jaeger EA, eds. *Duane's Ophthalmology*. 15th ed. Philadelphia, Pa: Lippincott Williams & Wilkins;2009:chap 5; Baloh RW, Jen J. Neuro-ophthalmology. In: Goldman L, Schafer AI, eds. *Cecil Medicine*. 24th ed. Philadelphia, PA: Saunders Elsevier; 2011:chap 432; Cleary TS, Reichel E. Electrophysiology. In: Yanoff M, Duker JS, eds. *Ophthalmology*. 3rd ed. St. Louis, Mo: Mosby Elsevier; 2008:chap 6.9.

[0049] Another measure of photoreceptor function that can be measured by retinography is a peak of electrical activity between 0.05 and 50 Hz following systemic introduction of sodium azide, known as the azide response.

Example 1: Preparation of SB623 cell suspensions

[0050] SB623 cells were obtained by transfection of human marrow adherent stem cells (MASCs) with DNA encoding the intracellular domain of the human Notch protein. MASCs were obtained from human bone marrow as follows. Human adult bone marrow aspirates were purchased from Lonza (Walkersville, MD). Cells were washed once, and plated in Corning T225 flasks (Corning, Inc. Lowell, MA.) in Growth Medium: alpha-MEM (Mediatech, Herndon, VA) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT), 2mM L-glutamine and penicillin/streptomycin (both from Invitrogen, Carlsbad, CA). After 3 days, unattached cells were removed; and the MASC cultures were maintained in growth medium for approximately 2 weeks. During that period, cells were passaged twice, using 0.25%Trypsin/EDTA.

[0051] To make SB623 cells, the MASCs were transfected with the pN-2 plasmid, which contains sequences encoding the human Notch1 intracellular domain (under the transcriptional control of the CMV promoter) and a neomycin-resistance gene (under the transcriptional control of a SV40 promoter), using Fugene6 (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. Briefly, cells were incubated with the Fugene6/plasmid DNA complex for 24 hours. The next day, medium was replaced with growth medium (components described above) containing 100 ug/ml G418 (Invitrogen, Carlsbad, CA), and selection was continued for 7 days. After removal of G418 selection medium, cultures were maintained in growth medium and expanded for 2 passages. SB623 cells were harvested using Trypsin/EDTA, formulated in freezing medium at cell densities of 7.5×10^3, 1.5×10^4 and 3×10^4 cells/ml and cryopreserved. Frozen SB623 cells were stored in the vapor phase of a liquid N_2 unit until needed.

Example 2: Intravitreal transplantation

[0052] RCS rats were immunosuppressed by administration of oral cyclosporine A (200 mg/l in drinking water) beginning at postnatal day 2 and continuing until transplantation. Transplantation of SB623 cells by injection occurred at four weeks after birth. Prior to transplantation, animals were systemically anesthetized with a mixture of xylazine hydrochloride (Celactal®, Bayer Medical, Ltd.) and ketamine hydrochloride (Ketalar®, Daiichi Sankyo Co., Ltd.) and topically

anesthetized with 0.4% oxybupurocaine hydrochloride (Benoxyl®, Santen Pharmaceutical Co., Ltd.). Pupils were dilated with tropicamide and phenylephrine hydrochloride (Mydrin-P®, Santen Pharmaceutical Co., Ltd.) prior to injection of 5 ul of SB623 cell suspension into the vitreous cavity. Injection was accomplished using a Hamilton syringe with a 30-gauge needle. Control cohorts were injected with vehicle (PBS) or were uninjected (naïve). The experimental design is shown in Table 1.

Table 1

Group	Treatment	Cell number (per eye)	Number of animals
1	Naïve	-	5
2	Vehicle (PBS)	-	5
3	SB623	3.75×10^4	5
4	SB623	7.5×10^4	5
5	SB623	1.5×10^5	7

[0053] Following transplantation of SB623 cells at 4 weeks of age, animals were tested at 5, 6, 8 and 12 weeks of age (*i.e.*, 1, 2, 4 and 8 weeks after transplantation) by electroretinography and at 12 weeks of age (8 weeks post-transplantation) for azide response. At 13 weeks of age (9 weeks after treatment), animals were sacrificed, and their eyes were removed for histological examination.

[0054] For electroretinography, rats were dark-adapted for one hour, then systemically anesthetized with a mixture of xylazine hydrocholride (Celactal®, Bayer Medical, Ltd.) and ketamine hydrochloride (Ketalar®, Daiichi Sankyo Co., Ltd.). Pupils were dilated with tropicamide and phenylephrine hydrochloride (Mydrin-P®, Santen Pharmaceutical Co., Ltd.). Electroretinograms (ERGs) were recorded with a contact electrode placed on the cornea and a grounding electrode placed in the nose. Responses were evoked with a white LED flash (3,162 cd/m^2, 10 ms duration) and recorded on a Neuropack S1 NEB9404 (Nihon Kohden Corp.).

[0055] Figure 1 (upper three pairs of panels) shows representative ERG traces, for vehicle-treated animals (left panels) and for animals treated with 1.5×10^5 SB623 cells per eye (right panels), obtained just prior to transplantation (at 4 weeks after birth), and at 4 and 8 weeks post-transplantation. Neither an a-wave nor a b-wave was observed in the vehicle-treated animals at 4- and 8-weeks post-treatment; while, in the SB623-treated animals, electrical activity was retained at these time points. A

quantitative assessment of receptor cell electrical activity, measured by ERG, is shown in Figure 2. At all time points tested, SB623-treated animals retained greater photoreceptor cell electrical activity that either naïve animals or vehicle-treated animals.

[0056] For determination of azide responses at 8 weeks post-transplantation, RCS rats were dark-adapted for one hour, then systemically anesthetized with a mixture of xylazine hydrocholride (Celactal®, Bayer Medical, Ltd.) and ketamine hydrochloride (Ketalar®, Daiichi Sankyo Co., Ltd.) and topically anesthetized with 0.4% oxybupurocaine hydrochloride (Benoxyl®, Santen Pharmaceutical Co., Ltd.). A contact electrode was placed on the cornea, and 0.1 ml of 0.1% sodium azide (NaN_3) was injected into the caudal vein. Responses were recorded on a Neuropack S1 NEB9404 (Nihon Kohden Corp.), amplified in the region between 0.05 and 50 Hz. Amplitudes were measured from baseline to the positive peak, which appeared approximately 4 seconds after injection of the azide solution.

[0057] The lower pair of panels in Figure 1 shows that the azide response was retained, at 8 weeks after treatment, in the eyes of RCS rats treated by intravitreal injection of 1.5×10^5 SB623 cells (lower right panel) but was lost in rats injected with PBS (lower left panel). Figure 3 shows measurements of the amplitude of the response in SB623-treated and control eyes. As shown, injection of 1.5×10^5 SB623 cells resulted in a statistically significant increase in the amplitude of the azide response at 8 weeks after treatment.

[0058] For histological analysis, rats were sacrificed, and their eyes were removed. After fixation in 4% paraformaldehyde, eyes were embedded in Technovit® 8100 resin (Heraeus Kulzer, Werheim, Germany) according to the manufacturer's instructions. Briefly, eyes were washed overnight at 4°C in PBS containing 6.8% sucrose, dehydrated in 100% acetone, and embedded in Cryomold® (EMS, Hatfield, PA). The polymerized block was fixed onto a wooden block with an adhesive agent and cut using a sliding microtome (HM440E, MICROM International GmbH, Walldorf, Germany) with a disposable knife. Three-micrometer sections were used for immunostaining with a human anti-mitochondrial antibody (Millipore MAB1273).

[0059] Histological analysis revealed that, in vehicle-treated eyes, most of the cells of the outer nuclear layer of the retina were absent by 9 weeks after treatment (Figure 4A). In contrast, in SB623-treated eyes, cells of the outer nuclear layer were well-preserved (Figure 4B). Clumps of transplanted SB623 cells were observed in the

vitreous body (Figures 5A and 5B) and a SB623 cell was also observed on the inner limiting membrane of the retina (Figures 5C and 5D). In additional experiments, it was observed that intravitreal transplantation of SB623 cells prevented loss of outer nuclear layer cells for up to 25 weeks after treatment, and that SB623 cells persisted in the vitreous body at this time.

[0060] The results of both electrophysiological and morphological analyses, presented above, indicate that intravitreal transplantation of SB623 cells preserved retinal function.

Example 3: Subretinal transplantation

[0061] SB623 cells were prepared as described in Example 1 and suspended in PBS to a density of 3 x 10^4 cells/ul. Immunosuppression of RCS rats, systemic and topical anesthesia, and dilation of pupils were all conducted as described in Example 2. Transplantation of SB623 cells occurred at four weeks after birth, by injection of 5 ul of SB623 cell suspension intravitreously into the subretinal space using a Hamilton syringe with a 30-gauge needle. Control cohorts were injected with vehicle (PBS) or were uninjected (naïve). The experimental design is shown in Table 2. In this experiment, analysis was continued for a longer period after treatment: electroretinography and azide response measurements were continued for 24 weeks, and histology and immunohistochemistry were conducted on specimens obtained 27 weeks after treatment.

Table 2

Group	Treatment	Cell number (per eye)	Number of animals
1	Naïve	-	4
2	Vehicle (PBS)	-	10
3	SB623	1.5 x 10^5	10

[0062] Electroretinography and determination of azide responses were conducted as described in Example 2. Representative results are shown in Figure 6. In most vehicle-treated rats, an ERG could not be recorded at 4 weeks after treatment (Figure 6, left panels). However, in SB623-treated animals, both ERGs and azide responses were retained at 24 weeks after treatment (Figure 6, right panels).

[0063] Figure 7 shows a time-course of changes in ERG amplitudes at four-week intervals up to 24 weeks post-transplantation. By 8 weeks after transplantation, neither an a-wave nor a b-wave could be detected in eyes from naïve and vehicle-treated rats; but in rats that had received a subretinal injection of SB623 cells, both a- and b-waves were retained up to 24 weeks post-treatment.

[0064] Figure 8 shows a time-course of changes in the azide response at four-week intervals up to 24 weeks post-transplantation. The response is reduced in naïve and vehicle-injected animals at all time points. In rats that had received a subretinal injection of SB623 cells, a statistically significant increase in azide response, compared to naïve and vehicle-injected rats was observed at all points up to 24 weeks post-treatment.

[0065] The results of these electrophysiological examinations indicate that transplantation of SB623 cells preserves retinal function for long-term periods.

[0066] To determine whether visual signals were transmitted from the retina to the visual cortex of the brain, visually evoked potentials (VEPs) were measured, in treated and untreated RCS rats, at 26 weeks after treatment. Seven days prior to VEP recording, screw electrodes were placed epidurally on each side of the head 6.8 mm behind the bregma and 3.2 mm lateral of the midline, and a reference electrode was placed epidurally on the midline 11.8mm behind the bregma. On the day of VEP recording, rats were dark-adapted for one hour, then systemically anesthetized with a mixture of xylazine hydrocholride (Celactal®, Bayer Medical, Ltd.) and ketamine hydrochloride (Ketalar®, Daiichi Sankyo Co., Ltd.). Pupils were dilated with tropicamide and phenylephrine hydrochloride (Mydrin-P®, Santen Pharmaceutical Co., Ltd.). VEP responses were evoked with a white LED flash (3,162 cd/m^2, 10 ms duration) and recorded on a Neuropack S1 NEB9404 (Nihon Kohden Corp.). One hundred responses were measured and the results were averaged. Representative results are shown in Figure 9. In naïve and vehicle-injected animals, VEPs could not be detected. In contrast, the VEP response was well-preserved, at 26 weeks after treatment, in rats that had been subretinally injected with SB623 cells. These results indicate that treatment with SB623 cells restores the ability to send visual signals to the visual cortex.

[0067] Histology and immunochemistry were conducted, as described in Example 2, on specimens obtained 27 weeks after treatment. As shown in Figure 10, by 27 weeks after transplantation, few if any cells of the outer nuclear layer (ONL)

were present in vehicle-treated rats. However, in SB623-treated rats, cells of the ONL were well-preserved at 27 weeks. In addition, transplanted SB623 cells, detected by immunostaining with anti-human mitochondrial antibody, were observed in the subretinal space (Figure 11).

[0068] These results demonstrate the long-term persistence of SB623 cells after subretinal injection, and show that the transplanted SB623 cells were able to prevent death of photoreceptor cells.

CLAIMS

What is claimed is:

1. Use of cells in the manufacture of a medicament for treating retinal degeneration in a subject in need thereof, wherein said cells are descendants of marrow adherent stem cells that have been engineered to express an exogenous Notch intracellular domain.

2. The use of claim 1, wherein said cells are to be transplanted into the eye of the subject.

3. The use of claim 2, wherein the transplantation is intravitreal.

4. The use of claim 2, wherein the transplantation is subretinal.

5. The use of any of claims 1 to 4, wherein the retinal degeneration occurs in retinitis pigmentosa.

6. The use of any of claims 1 to 4, wherein the retinal degeneration occurs in age-related macular degeneration (AMD).

7. The use of claim 1, wherein the retinal degeneration occurs in Usher syndrome, Stargardt's disease, Leber Congenital Amaurosis, choroidermia, Bardet-Biedl syndrome, or Refsum disease.

8. The use of claim 1, wherein the retinal degeneration occurs in Best's disease, cone-rod retinal dystrophy, gyrate atrophy, Oguchi disease, juvenile retinoschisis, Bassen-Kornzweig disease (abetalipoproteinemia), blue cone monochromatism disease, dominant drusen, Goldman-Favre vitreoretinal dystrophy (enhanced S-cone syndrome), Kearns-Sayre syndrome, Laurence-Moon syndrome, peripapillary choroidal dystrophy, pigment pattern dystrophy, Sorsby macular dystrophy, Stickler's syndrome or Wagner's syndrome (vitreoretinal dystrophy).

9. The use of claim 8, wherein the pigment pattern dystrophy is selected from the group consisting of Butterfly-shaped pigment dystrophy of the fovea, North Carolina macular dystrophy, macro-reticular dystrophy, spider dystrophy and Sjogren reticular pigment epithelium dystrophy.