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Stem Cell Therapy in Glaucoma

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ABSTRACT

Glaucoma is a chronic, degenerative optic neuropathy, which cause progressive damage to the optic nerve, retinal ganglion cell (RGC) death and characteristic damage to the visual field. Current glaucoma therapeutics lower intraocular pressure (IOP) but they do not repair the damaged optic nerve or reverse vision loss. So, new treatment strategies are in demand. Stem cell therapy presents a new intervention that holds great promise for reversing vision loss. There are at least 3 potential targets for stem cell therapy in glaucoma: the retinal ganglion cells , the optic nerve head, and the trabecular meshwork. Stem cells also have a neuroprotective effect in glaucoma by improving retinal ganglion cell survival. There are many obstacles in using stem cells in glaucoma as the regulation of differentiation, integration, host immune response, tumorigenesis and ethical concerns.

Keywords: glaucoma, stem cell, transplantation, cell therapy , trabecular meshwork, neuroprotection.

INTRODUCTION

Glaucoma is a chronic, degenerative optic neuropathy, associated with progressive degeneration of retinal ganglion cells, optic nerve damage and results in irreversible loss in the visual field. Current glaucoma therapeutics lower intraocular pressure by reducing aqueous humor formation or increasing outflow of fluid [(1)].

Stem cell therapy presents a new intervention that holds great promise for reversing vision loss. Particularly, as pluripotent stem cells can divide indefinitely and differentiate into almost any types of cells in the body. In addition, stem cells or progenitor cells can also work through a neuroprotective mechanism by secreting neurotrophic factors that prevent cell loss [(2)].

Glaucoma

Glaucoma is the leading cause of irreversible blindness in the world. It can remain asymptomatic until it is severe, resulting in presence of high number of undiagnosed individuals [(3)].

Glaucoma can be classified according to European Glaucoma Society Guidelines (EGSc) into:

1. Congenital forms/ childhood glaucoma.
2. Open angle glaucoma.
   a. Primary open angle glaucoma (POAG) : POAG with high pressure , POAG with normal pressure, Primary juvenile glaucoma, POAG suspect and Ocular hypertension.
   b. Secondary open angle glaucoma : Caused by ocular diseases, Iatrogenic secondary open angle glaucoma and Caused by extrabulbar disease.
3. Angle closure glaucoma.
   a. Primary angle closure glaucoma: Acute, Intermittent, Chronic, Status after acute angle closure glaucoma attack and Primary angle closure glaucoma suspect, and
   b. Secondary angle closure glaucoma: With pupillary block, With anterior pulling mechanism without pupillary block and With posterior pushing mechanism without pupillary block [(4)].

Aqueous Humor Dynamics

Aqueous humor is secreted by the ciliary body and fills the anterior chamber (the space between the cornea and the iris) and posterior chamber (the space between the iris and lens). There are two pathways for outflow of aqueous humor **,** the trabecular or conventional outflow pathway, through which most of the aqueous humor leaves the eye and uveoscleral pathway [(5)] .

Management of Glaucoma

Treatment of glaucoma is long term; thus, it needs to be supplemented by a comprehensive approach to the patient, including education about the condition and the purpose of treatment. All currently available drugs for treating glaucoma work by lowering the IOP via one or both of the following mechanisms: reduce the secretion of aqueous humor; and/or increase the outflow of aqueous humor through the trabecular meshwork and/or the uveoscleral pathway [(6)].

The treatment options currently available are medical therapy which is usually in the form of topical medications; laser therapy and surgery [(6)].

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Medications used in treatment of glaucoma can be topical as prostaglandin analogues, beta-blockers, alpha-adrenergic agonists, parasympathomimetic agents and topical carbonic anhydrase inhibitors or systemic as oral carbonic anhydrase inhibitors and hyperosmotic agents [7].

Laser trabeculoplasty reduces intraocular pressure by inducing biological changes in the trabecular meshwork resulting in increased aqueous drainage. The procedure has an excellent safety profile and is performed during an office visit. Although this intraocular pressure reduction can be achieved in the most of patients, the effect decreases gradually over time with a failure rate of about 10% per year [8].

Trabeculectomy is the most commonly performed incisional surgical procedure to reduce intraocular pressure. It consists of excision of a small portion of the trabecular meshwork and or adjacent corneoscleral tissue to provide a drainage route for aqueous humor from within the eye to underneath the conjunctiva where it is absorbed [9].

Basics of Stem Cells

Stem cells are certain biological cells found in all multicellular organisms; they represent a small fraction of body mass, but can divide through mitosis and differentiate into different specialized cell types and can self-renew to produce more stem cells [10].

CLASSIFICATION AND SOURCES OF STEM CELLS

Stem cells can be classified into four broad types based on their origin: stem cells from embryos, stem cells from the fetus , stem cells from the umbilical cord and stem cells from the adult, each of these can be grouped into subtypes [11].

Scientists have recently found another way of producing pluripotent stem cells without using embryos. They discovered how to 'reprogramme' some specialized cells to become pluripotent so that they lose their specialist functions and behave virtually in the same way as embryonic stem cells. Pluripotent cells generated in this way are called induced pluripotent stem cells (iPSCs) [12].

- **Embryonic stem cells**

Embryonic stem cells (ESCs) are derived from embryos, which developed from eggs that have been fertilized *in vitro* and then donated for research purposes with informed consent of the donors. They are not derived from eggs fertilized in a woman's body [13].

- **Fetal stem cells**

Fetal stem cells are primitive cell types found in the organs of fetuses. Neural crest stem cells, pancreatic islet progenitors and fetal hematopoietic stem cells have been isolated in abortuses. Fetal neural stem cells found in the fetal brain were shown to differentiate into both neurons and glial cells. Fetal blood, placenta, and umbilical cord are rich sources of fetal hematopoietic stem cells [14].

- **Umbilical cord stem cells**

Umbilical cord blood contains circulating stem cells and the cellular contents of umbilical cord blood appear to be quite distinct from those of adult peripheral blood and bone marrow. The frequency of umbilical cord blood hematopoietic stem cells equals or exceeds that of bone marrow and they are known to produce large colonies *in vitro*, have different growth factor requirements, long telomeres and can be expanded in long-term culture [15].

- **Adult tissue-derived stem cells**

Adult tissue-derived stem cells are found in many organs and tissues in the body, including bone marrow, intestine, blood, cornea, retina, skin, nervous system, brain and muscle. More precisely, they present in specific regions of an organ, the so-called 'stem cell niche'. Their role in the body is to replace cells which die throughout life due to wear and tear or injury and disease. For example, stem cells in bone marrow replace blood cells [16].

Potency of Stem Cells

The capacity to differentiate into specialized cell types and be able to give rise to any mature cell type is referred to potency. The potency of the stem cell specifies the differentiation potential i.e., the potential to differentiate into different cell types. Stem cells can be totipotent, pluripotent, mutipotent, oligopotent and unipotent [17].

Role of Stem Cells in Treatment of Glaucoma

Stem cell biology holds great promise to aid and expedite several major areas of glaucoma research and therapeutic development. These areas include studies of the possibility of RGCs regeneration by stem cells; restore the function of trabecular meshwork and stem cell mediated

neuroprotection. The development of stem cell-derived RGCs holds the potential to someday make possible the restoration of vision to patients who have already lost vision from optic nerve degeneration [18].

I- Regeneration of Retinal Ganglion Cells and Their Axons

One of the strategies to restore vision in glaucoma patients after RGCs have been lost or irreversibly damaged is their functional replacement by autologous or heterologous transplantation [19]. Different types of stem cells can be used for RGC therapies in glaucoma or other optic neuropathies, the most comprehensively studied donor cell candidate for cell-based therapies in the retina has been embryonic stem cells, which proliferate, self-renew and differentiate into all cell types [20].

- ***Human embryonic stem cells as a source of RGCs:***

Murine human embryonic stem cells have been shown to generate RGC-like cells *in vitro* by differentiation protocols using various growth and differentiating factors. This has resulted in the expression of markers such as Brn3b, RPF-1, Thy-1 and Isl-1which are characteristically expressed by RGCs [21].

Lamba et al. have provided evidence that human ESCs can generate retinal progenitors with high efficiency, expressing a number of molecular markers usually observed in the developing retina [22].

- ***Müller stem cells as a source of RGCs:***

Müller glia with stem cell characteristics have been demonstrated to be predominantly located in the peripheral sections of the adult human retina [23].

Singhal et al. reported differentiation of human Müller stem cells into RGCs precursors by stimulation with fibroblast growth factor 2 together with NOTCH inhibition using DAPT. Differentiation into RGC precursors was confirmed by gene and protein expression analysis, changes in cytosolic [Ca2] in response to neurotransmitters, and green fluorescent protein (GFP) expression by cells transduced with a transcriptional BRN3b, GFP reporter vector [24].

- ***Induced pluripotent stem cells as a source of RGCs :***

Some of the disadvantages of human ESCs have been addressed by the development of iPSCs, which have been proposed as a viable source of cells for autologous transplantation. The generation of iPSCs does not require the destruction of embryonic tissue and therefore does not have the same ethical implications as work with ESCs [25].

Parameswaran et al. have provided evidence that iPSCs, which originated from reprogrammed mouse embryonic fibroblasts by transfection with Oct3/4, Sox2, Klf4 and c- Myc, can give rise to both RGCs and photoreceptors *in vitro* [26].

II- Restore the Function of the Trabecular Meshwork

The trabecular meshwork (TM) is a wedge-shaped lamellar tissue stretched between the periphery of Descemet's membrane of the cornea anteriorly and the scleral spur posteriorly, it consists of the inner uveal meshwork, the deeper corneoscleral meshwork and the juxtacanalicular meshwork [27].

In addition to the 3 components of the TM in the outflow facility, there is proof of a fourth region called the insert region. The insert region resides at the Schwalbe's line and does not filter aqueous humor into the Schlemm's canal [28].

Gonzalez et al. cultured human TM cells from the insert region as free-floating spheres and found that these spheres could be grown for more than 3 months expressing neural precursor marker nestin, as well as leukemia inhibitor factor. They concluded that the spheres may contain relatively undifferentiated cells derived from human TM [29].

Bone marrow-derived mesenchymal stem cells (MSCs) are multipotent and have been explored for TM regeneration. In the study by Manuguerra-Gagne et al. MSCs were injected into the anterior chamber of rats with laser-induced glaucoma. MSCs injection induced a rapid return to normal IOP levels in experimental glaucomatous rats [30].

Abu-hassan et al. used TM cell–derived extra cellular matrix (ECM) and TM cell–derived conditioned media to achieve TM differentiation from human iPSCs. In their encouraging study, Abu-Hassan et al. further reported that when transplanted into an ex vivo human TM cell loss model, the iPSC derived TM like cells resembled endogenous TM cells and successfully restored, at least partially, IOP homeostatic function [31].

III- Stem Cell Mediated Neuroprotection

Generalized optic nerve axonal transport impairment and blockade of neurotrophic factor (NTF)-specific transport in the optic nerve, with relative NTF deprivation to the retina, has been observed in glaucoma models of numerous species [(32)]. Stem cells are able to facilitate local neuronal survival by the production of several NTF. This multifactorial effect has been demonstrated in animal models of CNS disease and work in a rodent model of Parkinson's disease showed that neural stem cell transplantation conferred a more significant neuroprotective benefit than both a single injection of neurotrophins or prolonged delivery via local infusion [(33)].

Johnson et al. showed the neuroprotective effects of MSCs and MSC-derived factors in organotypic retinal explant culture and an in vivo model of ocular hypertensive glaucoma. Co-culture of rat and human bone marrow-derived MSCs with retinal explants increased retinal ganglion cell survival [(34)].

Challenges in Treatment of Glaucoma by Stem Cells

Although some progress has been made regarding the successful production, delivery, integration and survival of RGC progenitors, major obstacles for successful engraftment, functional restoration and ethical problems remain. Researchers try to overcome each of these barriers to make stem cell therapy meaningfully, reliably, and safely restore vision and improve quality of life [(35)].

The ideal stem cell transplantation would be readily available, easy to expand in culture, safe in the long term, and autologous so as to avoid the host immune response. It would also have the ability to differentiate reliably into the desired target cell. At their current stage of development, many stem cell sources hold promise, but each also has critical flaws [(36)].

Tumorigenesis is a potential complication of every form of stem cell transplantation, but has been described most frequently with ESCs and iPSCs. Their capacity for unlimited renewal and differentiation, which makes them so promising as a cell replacement therapy, also causes them to have the greatest risk for uncontrolled proliferation and tumor formation. Many of the gene networks that are responsible for the replicative potential of ESCs and iPSCs are oncogenic in nature [(37)].

CONCLUSION

The use of stem cells in the clinical therapy of glaucoma will be an important step in the future as it will transform present-day treatment with the hope of restoring sight to patients with glaucoma. It is important to recognize that whatever contribution stem cell transplantation may make in the future to the management of glaucoma, it must be carried out in conjunction with IOP reduction. Combination therapies are likely to be efficacious in preventing vision loss in glaucoma, and ideally multiple, synergistic avenues for glaucoma treatment will be available in the future. Moreover, even if RGC replacement is one day feasible, IOP must be effectively and continually managed to prevent glaucomatous degeneration of the replacement RGCs.

REFERENCES

1. **Cherecheanu AP, Garhofer G, Schmidl D *et al.* (2013):** Ocular perfusion pressure and Ocular blood flow in glaucoma. Curr Opin Pharmacol., 13:36–42.
2. **Doorenbos M, Lu S, Chen DF (2015):** Development of a Stem Cell Therapy for Glaucoma. US Ophthalmic Review, 8(1):52–54.
3. **Leite MT, Sakata LM, Medeiros FA (2011):** Managing glaucoma in developing countries. Arq Bras Oftalmol., 74(2):83–84.
4. **European Glaucoma Society (EGSc) (2014):** Terminology and Guidelines for Glaucoma. 4th ed. Savona, Italy.
5. **American Academy of Ophthalmology (AAO) (2011):** The Eye M.D. Association. In: 2011-2012 Basic and Clinical Science Course, Section 10: Glaucoma. San Francisco, CA: American Academy of Ophthalmology.
6. **Barton K, Hitchings RA (2013):** Medical Management of Glaucoma.Springer Healthcare,pp.71-100.
7. **Lee DA, Higginbotham EJ (2005):** Glaucoma and its treatment: a review. Am J Health Syst Pharm., 62:691- 699.
8. **Dietlein TS, Hermann MM, Jordan JF (2009):** The medical and surgical treatment of glaucoma .Dtsch Arztebl Int., 106(37): 597–606.
9. **Gedde SJ, Schiffman JC, Feuer WJ *et al*. (2012):** Treatment outcomes in the Tube Versus Trabeculectomy (TVT) study after five years of follow-up. Am J Ophthalmol., 153(15):7892–8032.
10. **Weissman IL (2000):** Stem cells: units of development units of regeneration and units in evolution. Cell, 100: 157–168.

11. **Anderson DJ, Gage FH, Weissman IL (2001):** Can stem cells cross lineage boundaries? Nature, **7**: 393–395.
12. **Takahashi K, Yamanaka S (2006):** Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126 (4): 663-76.
13. **Binas B (2009):** Embryo-derived stem cells - a system is emerging. BMB Reports, 42 (2): 72-80.
14. **Brustle O, Choudary K, Karram K *et al.* (1998):** Chimeric brains generated by intraventricular transplantation of human brain cells into embryonic rats. Nat Biotech., 16: 1040–1044.
15. **Rogers I, Casper RF (2004):** Umbilical cord blood stem cells. In : N. Fisk & J. Itskovitz eds. Best Practice & Research Clinical Obstetrics& Gynaecology , London: Elsevier Ltd. pp. 893–908.
16. **Walker MR, Patel KK, Stappenbeck TS (2009):** The stem cell niche. Journal of Pathology, 217 (2): 169-80.
17. **Hima B A, Srilatha B (2011):** Potency of Various Types of Stem Cells and their Transplantation. J Stem Cell Res Ther ., 1:115.
18. **Johnson TV, Bull ND, Martin KR (2011):** Stem cell therapy for glaucoma: possibilities and practicalities. Expert Rev Ophthalmol., 6(2): 165–74.
19. **Kerrigan-Baumrind LA, Quigley HA, Pease ME *et al.* (2000):** Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons. Invest Ophthalmol Vis Sci .,41: 741-748.
20. **Meyer JS ,Shearer RL, Capowski EE *et al.* (2009):** Modeling early retinal development with human embryonic and induced pluripotent stem cells. Proc Natl Acad Sci USA, 106(39):16698–16703.
21. **Jagatha B, Divya MS, Sanalkumar R *et al.* (2009):** In vitro differentiation of retinal ganglion-like cells from embryonic stem cell derived neural progenitors. Biochem Biophys Res Commu., 380:230-235.
22. **Lamba DA, Karl MO, Ware CB *et al.* (2006):** Efficient generation of retinal progenitor cells from human embryonic stem cells. Proc Natl Acad Sci U S A, 103: 12769-12774.
23. **Bhatia B, Singhal S, Lawrence JM *et al.* (2009):** Distribution of Muller stem cells within the neural retina: evidence for the existence of a ciliary margin like zone in the adult human eye. Exp Eye Res., 89: 373-382.
24. **Singhal S, Bhatia B, Jayaram H *et al.* (2012):** Human Müller Glia with Stem Cell Characteristics Differentiate into Retinal Ganglion Cell (RGC) Precursors In Vitro and Partially Restore RGC Function In Vivo Following Transplantation. STEM CELLS TRANS MED., 1:188–199.
25. **Wu N, Doorenbos M, Chen DF (2016):** Induced Pluripotent Stem Cells: Development in the Ophthalmologic Field. Stem Cells International, (1): 2361763.
26. **Parameswaran S, Balasubramanian S, Babai N *et al.* (2010):** Induced pluripotent stem cells generate both retinal ganglion cells and photoreceptors: therapeutic implications in degenerative changes in glaucoma and age-related macular degeneration. Stem Cells, 28: 695-703.
27. **Tamm ER (2009):** The trabecular meshwork outflow pathways: structural and functional aspects. Exp. Eye Res., 88:648– 655.
28. **Yun H, Zhou Y, Wills A *et al.* (2016):** Stem Cells in the Trabecular Meshwork for Regulating Intraocular Pressure. Journal of Ocular Pharmacology and Therapeutics, 32(5): 253-260.
29. **Gonzalez P, Epstein DL, Luna C *et al.* (2006):** Characterization of free- floating spheres from human trabecular meshwork (HTM) cell culture in vitro. Exp Eye Res., 82:959–967.
30. **Manuguerra-Gagne R, Boulos PR, Ammar A *et al.* (2013):** Transplantation of mesenchymal stem cells promotes tissue regeneration in a glaucoma model through laser-induced paracrine factor secretion and progenitor cell recruitment. Stem Cells, 31:1136–1148.
31. **Abu-Hassan DW, Li X, Ryan EI, Acott TS *et al.* (2015):** Induced pluripotent stem cells restore function in a human cell loss model of open-angle glaucoma. Stem Cells, 33:751–761.
32. **Iwabe S, Moreno-Mendoza NA, Trigo-Tavera F *et al.* (2007):** Retrograde axonal transport obstruction of brain-derived neurotrophic factor (BDNF) and its TrkB receptor in the retina and optic nerve of American Cocker Spaniel dogs with spontaneous glaucoma. Vet. Ophthalmol., 10 (1):12–19.
33. **Yasuhara T, Matsukawa N, Hara K *et al.* (2006):** Transplantation of human neural stem cells exerts neuroprotection in a rat model of Parkinson's disease. J Neuro sci., 26: 12497-12511.
34. **Johnson TV, DeKorver NW, Levasseur VA *et al.* (2014):** Identification of Retinal Ganglion Cell Neuroprotection Conferred by Platelet-Derived Growth Factor through Analysis of the Mesenchymal Stem Cell Secretome Brain, 137(2) :503–519.
35. **Dahlmann-Noor AH, Vijay S, Limb GA *et al.* (2010):** Strategies for optic nerve rescue and regeneration in glaucoma and other optic neuropathies. Drug Discov Today, 15(7–8): 287–299.
36. **Jalving M, Shepers H (2009):** Induced pluripotent stem cells: will they be safe? Curr Opin Mol Ther., 11: 383-393.
37. **Ben-David U, Benvenisty N (2011):** The tumorigenicity of human embryonic and induced pluripotent stem cells. Nat Rev Cancer, 11:268-77.