PERSPECTIVE

Stem cell therapy for retinal ganglion cell degeneration

The prospects of stem cell therapy for retinal ganglion cell (RGC) degeneration in human: RGC degeneration is a common pathologic cause of glaucoma and optic neuropathies, which are the leading cause of irreversible blindness and visual impairment in developed countries, currently affecting more than 100 million people worldwide. Intraocular pressure lowering can slow down glaucoma progression in a proportion of patients. Also, there is still no effective therapy for optic neuropathies. Besides, the degenerated RGCs in glaucoma cannot be repaired, and human retina has limited regenerative potential. Therefore, the development of new therapeutic treatments against RGC degeneration is needed. Cell replacement and neuroprotection are the principle strategies for glaucoma and optic neuropathy treatment. Replacing the diseased or degenerated cells by stem cell-derived RGCs should provide effective therapeutic treatment. However, complex circuitry in the retina makes cell replacement challenging and difficult for functional repair. Alternatively, neuroprotection is more realistic and applicable to preserve the patients' vision. Numerous neuroprotection strategies have been investigated, including peripheral nerve grafting, electrical stimulation, application of neurotrophic factors (brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial cell-derived neurotrophic factor (GDNF) and nerve growth factor (NGF), direct intrinsic regeneration stimulation, RNA interference and human adult stem cells. Our group recently reported that the intravitreal transplantation of human periodontal ligament-derived stem cells (PDLSCs) ameliorates RGC degeneration after optic nerve injury in rats and promotes neural repair by enhancing axon regeneration through cell-cell interaction and neurotrophic factor secretion from PDLSCs (Cen et al., 2018). At present, there are 9 clinical trials on human adult stem cells for glaucoma and optic nerve diseases (www.clinicaltrials.gov/; **Table 1**). An emerging role of human adult stem cell therapy for glaucoma and optic neuropathy treatments is foreseeable in the near future.

Human adult stem cells for RGC protection: Adult stem cells are the quiescent undifferentiated cells found in fully developed tissues with the abilities to self-renew and differentiate into mature cells. Adult stem cells can be conveniently isolated from accessible tissues, including bone marrow, peripheral blood, adipose tissues and teeth. Different types of adult stem cells can be identified according to their lineages, such as hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and neural stem cells. Although adult stem cells function to maintain the adult tissue homeostasis by cell replacement and tissue regeneration, they can also modulate the microenvironment in the host tissue and protect the RGCs from degeneration.

The neuroprotective effect of adult stem cells for RGC degeneration has been mainly studied in MSCs. There is no reported study on RGC protection by HSCs. Different sources of MSCs, including rat and mouse bone marrow, adipose tissue, human chorionic plate and rat dental pulp, have been shown to enhance RGC survival after optic nerve injury (Mead et al., 2013; Chung et al., 2016; Hu et al., 2017; Li et al., 2018). The mechanisms of MSC neuroprotection can be modulating the plasticity of damaged host tissues, secreting neurotrophic and survival-promoting growth factors, restoring synaptic transmitter release, integrating into existing neural and synaptic networks, and re-establishing functional afferent and efferent connections (Ng et al., 2014). In a secretome study, 25 secreted proteins were identified from human bone marrow-derived MSCs (Johnson et al., 2014), among which interferon-γ, interleukin (IL)-11, leukemia inhibitory factor, IL-6, BDNF, platelet derived growth factor AA (PDGF-AA) and PDGF-AB/BB are enriched in human bone marrow-derived MSCs compared to human adult dermal fibroblasts. Moreover, secretion of NGF, BDNF and neurotrophin-3 (NT-3) was reported in rat dental pulp-derived stem cells (Mead et al., 2013). BDNF and GDNF are important regulators in neuroprotection of mouse bone marrow-derived MSCs (Hu et al., 2017). In our study, we found that human PDLSCs after intravitreal transplantation can survive and migrate to the RGC layer and even to the optic nerve (Cen et al., 2018). The cell-cell interaction is a critical condition to protect RGCs from degeneration since RGC survival is increased in the contact fashion of human PDLSC-retinal explant co-culture. In addition, human PDLSCs highly express BDNF, CNTF, GDNF and NT-3, which are the essential neurotrophic factors to enhance RGC survival and axon regeneration. Notably, we discovered a novel mechanism of human adult stem cells that the injured retina enhances BDNF secretion from human PDLSCs (**Figure 1**). How this positive feedback stimulated by the host retinal injury enhances BDNF secretion from human PDLSCs and what factors and retinal cell types are involved in this stimulation require further investigations. Besides, human PDLSC transplantation induces mild inflammation in rats, and inflammation has been reported to promote neural survival and axon regeneration in return. Whether the induction of mild inflammation by the xeno-transplantation contributes to the increased RGC survival and axon regeneration remains to be delineated. Nevertheless, results

Table 1 Registered adult stem cell-based clinical trials for glaucoma and optic nerve diseases

Identifier	Sponsor	Country	Status	Study	Phase of trial	Cell type	Delivery	Estimated number of patients	Estimated trial end
NCT01364246	Shenzhen Beike Bio-Technology Co., Ltd.	China	Unknown	Safety and efficacy of umbilical cord mesenchymal stem cell therapy for patients with progressive multiple sclerosis and neuromyelitis optica	Phase 1/2	Umbilical cord mesenchymal stem cells		20	2014
NCT01834079	Chaitanya Hospital, Pune	India	Unknown	Study the safety and efficacy of bone marrow derived autologous cells for the treatment of optic nerve disease	Phase 1/2	Bone marrow derived autologous mono nuclear cells	Intrathecal	24	2016
NCT01920867	MD Stem Cells	United States and United Arab Emirates	Enrolling by invitation	Stem cell ophthalmology treatment study (SCOTS)		Autologous bone marrow-derived stem cells	Retrobulbar, Subtenon, Intravenous, Intravitreal, Intraocular	300	2019
NCT02144103	Burnasyan Federal Medical Biophysical Center	Russian Federation	Enrolling by invitation	Effectiveness and safety of adipose-derived regenerative cells for treatment of glaucomatous neurodegeneration	Phase 1/2	Autologous adipose-derived regenerative cells	Subtenon	16	2017
NCT02249676	Tianjin Medical University General Hospital	China	Unknown	Autologous mesenchymal stem cells for the treatment of neuromyelitis optica spectrum disorders	Phase 2	Autologous mesenchymal stem cells	Intravenous	15	2014
NCT02330978	University of Sao Paulo	Brazil	Recruiting	Intravitreal Mesenchymal stem cell transplantation in advanced glaucoma	Phase 1	Autologous bone marrow-derived mesenchymal stem cells	Intravitreal	12	2016
NCT02638714	Stem Cells Arabia	Jordan	Unknown	Treatment of optic neuropathies using autologous bone marrow-derived stem cells	Phase 1/2	Autologous bone marrow-derived stem cells		100	2018
NCT03011541	MD Stem Cells	United States	Recruiting	Stem cell ophthalmology treatment study II (SCOTS2)		Autologous bone marrow-derived stem cells	Retrobulbar, Subtenon, Intravenous, Intravitreal, Intraocular	500	2021
NCT03173638	Instituto Universitario de Oftalmobiología Aplicada	Spain	Not yet recruiting	Safety Assessment of Intravitreal Mesenchymal stem cells for acute non arteritic anterior ischemic optic neuropathy	Phase 2	Allogenic mesenchymal stem cells	Intravitreal	5	2019

Information obtained from http://clinicaltrials.gov/ (March 23rd, 2018).

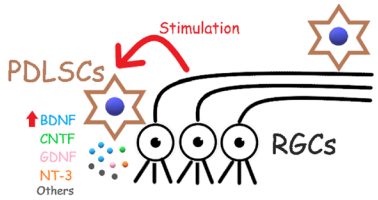

Figure 1 The retinal ganglion cell (RGC) protective mechanisms of human periodontal ligament-derived stem cells (PDLSCs).
Human PDLSCs after intravitreal transplantation can survive and migrate to the RGC layer and even to the optic nerve. The RGC protective mechanisms of human PDLSCs are mediated by cell-cell interaction and neurotrophic factor secretion (brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial cell-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3) and others) by human PDLSCs. The injured retina can positively feedback to human PDLSCs to enhance their secretion of BDNF.

of our human PDLSCs study and other reported studies indicate a potential clinical application of MSCs for glaucoma and optic neuropathy treatment in future.

Human adult stem cells for RGC regeneration: The basis of cell replacement therapy is that new RGCs could be regenerated from stem cells to substitute the damaged RGCs in glaucoma or optic neuropathies. Pluripotent stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have been studied for their differentiation potential into retinal lineages (Eiraku et al., 2011). Although adult stem cells are believed to be tissue-specific and only possess restricted differentiation capability, increasing number of studies report that adult stem cells are capable of giving rise to cells to an entirely distinct lineage. Our group have previously demonstrated that human PDLSCs can be induced to retinal lineage (Huang et al., 2013) and generate electrically functional RGC-like cells (Ng et al., 2015). Notably, pluripotent sub-population can be found in human PDLSCs (Pelaez et al., 2013). These pluripotent adult stem cells are the neural crest stem cells residing in neural crest-derived adult tissues. They can form teratomas in the immunodeficient mice with tissues from the three embryological germ layers (endoderm, mesoderm, and ectoderm), and can be induced into neuronal lineage, implying that pluripotent adult stem cells can be isolated and enriched from human adult tissues without the necessity of reprogramming. The efficiency of RGC production can be enhanced with the use of pluripotent adult stem cells. Adult stem cells, in addition to the ESCs and iPSCs, can also be used to produce RGCs for glaucoma and optic neuropathy treatments. Nevertheless, the replacement therapy based on exogenous stem cell-derived RGCs remains challenging because of the complex circuitry of the inner retina and the precise axonal projection to brain targets.

Another possible strategy for RGC regeneration is the endogenous regeneration by retinal stem cells. Unlike the limbal stem cells for corneal epithelium regeneration, retinal stem/progenitor cells are hardly identified in adult high-order mammalian retina. In contrast, Müller glia can be transiently reprogrammed into retinal progenitor stage for endogenous regeneration through de-differentiation and re-differentiation. However, the endogenous activation of Müller glia is extremely low under normal circumstances. Müller glia reprogramming can be enhanced by retinal damage and induction of Wnt/β-catenin signalling. The reprogrammed Müller glia can be stably maintained by fusion with exogenously transplanted or endogenous adult stem cells through cell-hybrid formation (Sanges et al., 2013). The reprogrammed Müller glia can proliferate and differentiate into RGCs and amacrine cells in the N-methyl-D-aspartic acid-treated mouse retina. Further work is needed to determine the treatment efficacy in other RGC degeneration models and to refine the differentiation signals for specific RGC regeneration from the de-differentiated Müller glia *in vivo*.

Conclusion and future perspective: Endogenous regeneration by retinal stem cells is the best regimen for RGC replacement against the RGC degenerative diseases. However, due to their limited availability, stem cell-based treatment relies on exogenous stem cell sources. Among different types of stem cells, MSCs are excellent for transplantation since they possess strong immunosuppressive properties and inhibit the release of pro-inflammatory cytokines, allowing autologous as well as allogeneic transplantation without the need of pharmacological immunosuppression. Moreover, MSCs can be transplanted directly without genetic modification or pre-treatments, and are able to migrate to the tissue injury sites without the concern of teratoma formation after transplantation. No moral objection or ethical controversies involved in their attainment. Importantly, MSCs can be directly applied for neuroprotection, and they can also be induced to neuronal cells for replacement therapy. These biological properties and the expansion potential of MSCs provide the therapeutic applications of MSCs to treat different human diseases, especially the RGC degenerative diseases. Yet, there are queries and uncertainties. Which MSC sources and types are optimal for neuroprotection? Can the neuroprotective effect of MSCs be modulated and enhanced? What is the optimal cell number and stage for transplantation? Which transplantation route is suitable for each individual optic neuropathy? Further research is needed to optimize and standardize the stem cell treatment effect before the routine clinical application of stem cell therapy for RGC degenerative diseases.

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