


# Diversified Treatment Options of Adult Stem Cells for Optic Neuropathies

Cell Transplantation  
Volume 31: 1–21  
© The Author(s) 2022  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/09636897221123512  
journals.sagepub.com/home/ctl  


Shaoying Tan<sup>1,2,3\*</sup>, Yao Yao<sup>1,4\*</sup>, Qichen Yang<sup>5\*</sup>, Xiang-Ling Yuan<sup>1,4\*</sup>,  
Ling-Ping Cen<sup>1</sup>, and Tsz Kin Ng<sup>1,4,5</sup> 

## Abstract

Optic neuropathies refer to a group of ocular disorders with abnormalities or dysfunction of the optic nerve, sharing a common pathophysiology of retinal ganglion cell (RGC) death and axonal loss. RGCs, as the retinal neurons in the central nervous system, show limited capacity in regeneration or recovery upon diseases or after injuries. Critically, there is still no effective clinical treatment to cure most types of optic neuropathies. Recently, stem cell therapy was proposed as a potential treatment strategy for optic neuropathies. Adult stem cells, including mesenchymal stem cells and hematopoietic stem cells, have been applied in clinical trials based on their neuroprotective properties. In this article, the applications of adult stem cells on different types of optic neuropathies and the related mechanisms will be reviewed. Research updates on the strategies to enhance the neuroprotective effects of human adult stem cells will be summarized. This review article aims to enlighten the research scientists on the diversified functions of adult stem cells and consideration of adult stem cells as a potential treatment for optic neuropathies in future clinical practices.

## Keywords

adult stem cells, optic neuropathies, retinal ganglion cells, neuroprotection, neuronal differentiation

## Introduction

Optic neuropathies refer to a group of disorders with abnormalities and dysfunction of the optic nerve. Apart from the primary optic nerve disorders, some optic neuropathy cases are associated with systemic disorders, such as demyelinating inflammatory optic neuritis (ON), which are associated with multiple sclerosis or neuromyelitis optic neuropathy. Other types of inflammatory ON can be associated with systemic autoimmune disorders, such as systemic lupus erythematosus or sarcoidosis<sup>1,2</sup>. Ischemic optic neuropathy is suggested to be associated with other conditions, including hypertension and diabetes. Tumors involving the optic nerves can co-exist with the tumors in the central nervous system. Different types of optic neuropathies can lead to various severities of visual impairment and even blindness. Acute and rapid visual deterioration is typical for demyelinating, inflammatory, ischemic, and traumatic causes, while a gradual course indicates the compressive, toxic/nutritional, or hereditary causes. Central scotoma and centrocecal scotoma are usually found in hereditary optic neuropathy. Altitudinal defects present in ischemic optic neuropathy and hemianopia can be associated with compressive optic neuropathy. Glaucoma, the main type of irreversible optic neuropathy, begins without apparent symptoms in the early stages but with initial gradual deterioration of peripheral vision<sup>3</sup>.

The classification and characteristics of optic neuropathies were summarized in Table 1.

Optic neuropathies can also be classified as acquired or inherited<sup>4</sup>. Acquired optic neuropathies affect people with wide spectrum of ages, but commonly occur in adults. The morphological changes involve not only the optic nerve head but also the entire optic nerve and blood vessels, and even co-existing with the intracranial problems. Their etiologies

<sup>1</sup> Joint Shantou International Eye Center of Shantou University and The Chinese University of Hong Kong, Shantou, China

<sup>2</sup> School of Optometry, The Hong Kong Polytechnic University, Kowloon, Hong Kong

<sup>3</sup> Research Centre for SHARP Vision, The Hong Kong Polytechnic University, Kowloon, Hong Kong

<sup>4</sup> Shantou University Medical College, Shantou, China

<sup>5</sup> Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Kowloon, Hong Kong

\*These authors contributed equally to this work.

Submitted: April 21, 2022. Revised: July 28, 2022. Accepted: August 16, 2022.

## Corresponding Author:

Tsz Kin Ng, Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong, North Dongxia Road, Shantou 515041, Guangdong, China.

Email: micntk@hotmail.com



**Table 1. Characteristics of Representative Optic Neuropathies and Current Adult Stem Cell Applications.**

Classification	Disease	Descriptions	Prevalence	Treatments	Adult stem cell applications
Inflammatory	<ul style="list-style-type: none"> <li>Demyelinating optic neuritis</li> </ul>	<ul style="list-style-type: none"> <li>Early sign of neuromyelitis optica spectrum disorder (NMOSD), multiple sclerosis (MS), or other autoimmune disorders on the spinal cord, brain, and optic nerve<sup>4</sup></li> <li>Anti-aquaporin-4 (AQP4) and anti-myelin oligodendrocyte glycoprotein (MOG) auto-antibodies can be discovered<sup>5-7</sup></li> <li>Exist subtype of no specific antibody identified<sup>7</sup></li> <li>Non-inflammatory disease of short posterior ciliary artery</li> <li>Coincidence of cardiovascular risk factors in a patient with "crowded" optic discs</li> </ul>	<ul style="list-style-type: none"> <li>Affecting young adults ranging from 18-45 years of age, with a mean age of 30-35 years</li> <li>Strong female predominance</li> <li>Approximate annual incidence of 5/100,000, with an estimated prevalence of 115/100,000<sup>8</sup></li> </ul>	<ul style="list-style-type: none"> <li>Intravenous injection methylprednisolone is used to treat optic neuritis in clinic<sup>2,10</sup></li> <li>Plasmapheresis and monoclonal antibody are supplied to neuromyelitis optica (NMO)<sup>11-13</sup></li> </ul>	<ul style="list-style-type: none"> <li>Improvement in visual acuity and other symptoms and signs by autologous peripheral HSC<sup>14</sup> or human umbilical cord-derived MSC transplantation<sup>5,16</sup> in NMO patient.</li> <li>Improvement in visual acuity by autologous bone marrow-derived MSC transplantation in patients with idiopathic bilateral ON<sup>17</sup>, recurrent ON<sup>18</sup> and autoimmune ON<sup>19</sup></li> </ul>
Ischemic <sup>20</sup>	<ul style="list-style-type: none"> <li>Non-arteritic anterior ischemic optic neuropathy</li> </ul>	<ul style="list-style-type: none"> <li>Non-inflammatory disease of short posterior ciliary artery</li> <li>Coincidence of cardiovascular risk factors in a patient with "crowded" optic discs</li> </ul>	<ul style="list-style-type: none"> <li>2.3 and 110.3 per 100,000 population per year<sup>21</sup>.</li> </ul>	<ul style="list-style-type: none"> <li>Aspirin prevents contralateral eye from non-arteritic anterior ischemic optic neuropathy<sup>22,23</sup></li> <li>The effect of corticosteroid on non-arteritic anterior ischemic optic neuropathy remains controversial<sup>24</sup></li> <li>Intravenous injection methylprednisolone</li> </ul>	<ul style="list-style-type: none"> <li>Transplantation of adult bone marrow cells induces retinal remodeling after ischemic injury by cellular regeneration and supporting endogenous repair<sup>25</sup>.</li> </ul>
Hereditary	<ul style="list-style-type: none"> <li>Arteritic anterior ischemic optic neuropathy</li> <li>Posterior ischemic optic neuropathy (PION)</li> <li>Leber hereditary optic neuropathy (LHON)</li> <li>Dominant optic atrophy</li> <li>Other hereditary optic neuropathies</li> <li>Nutritional and toxic optic neuropathy</li> </ul>	<ul style="list-style-type: none"> <li>Temporal arteritis (also called giant-cell arteritis)</li> <li>Inadequate blood flow (ischemia) to retrobulbar portion of the optic nerve</li> <li>Mitochondrial inherited (transmitted from mother to offspring) degeneration of RGCs and their axons</li> <li>Pathogenic mitochondrial DNA point mutations in the genes of complex I of oxidative phosphorylation chain in mitochondria</li> <li>Acute or sub-acute loss of central vision</li> <li>Inherited in an autosomal dominant manner (such as OPA1 mutations) and may be caused by a mutation in any of several genes<sup>30</sup></li> <li>Optic nerve damage secondary to a toxic substance and/or nutritional deficiency</li> </ul>	<ul style="list-style-type: none"> <li>About 8,000 individuals per year in United States<sup>26</sup></li> <li>PION most commonly affects the elderly</li> <li>In Northern European populations, about one in 9,000 people carry one of the three primary LHON mutations<sup>27,28</sup></li> <li>Prevalence between 1:30,000 and 50,000 in Europe</li> <li>Commonly vary between 1/10,000 in Denmark (due to a founder effect) to 1/35,000 in United Kingdom<sup>31</sup></li> <li>Relatively uncommon and is primarily associated with specific medications, occupational exposures, or tobacco and alcohol abuse</li> </ul>	<ul style="list-style-type: none"> <li>Currently no effective treatment</li> <li>Gene and stem cell therapies could be benefit for visual acuity improvement</li> </ul>	<ul style="list-style-type: none"> <li>Improvement in visual acuity and peripheral vision by transplantation of autologous bone marrow-derived stem cells in LHON patients<sup>29</sup>.</li> </ul>
Nutritional and toxic					

(continued)

**Table 1. (continued)**

Classification	Disease	Descriptions	Prevalence	Treatments	Adult stem cell applications
Papilloedematous	<ul style="list-style-type: none"> <li>Papilloedema</li> </ul>	<ul style="list-style-type: none"> <li>Secondary to raised intracranial pressure</li> </ul>	<ul style="list-style-type: none"> <li>Estimated annual incidence of 0.9 per 100,000 individuals in general population and 3.5 per 100,000 in females of 15–44 years in United States<sup>32</sup>.</li> </ul>		
• Traumatic	<ul style="list-style-type: none"> <li>Traumatic optic neuropathy</li> </ul>	<ul style="list-style-type: none"> <li>Direct head and facial trauma</li> <li>Indirect head and facial trauma</li> </ul>	<ul style="list-style-type: none"> <li>0.5 to 5% for indirect TON in closed-head trauma<sup>33</sup></li> </ul>	<ul style="list-style-type: none"> <li>Use of steroids, which is controversial, as the commonly treatment</li> <li>Decompression of optic nerve canal is recommended when suppression occurs to the optic nerve canal<sup>34</sup></li> </ul>	<ul style="list-style-type: none"> <li>Intravitreal injection of rat bone marrow-derived stem cells increases RGC survival and promotes axonal regeneration in rat model<sup>35</sup>.</li> <li>Intravitreal injection of human PDLSCs promotes RGC survival and axonal regeneration after optic nerve injury<sup>12</sup>.</li> <li>Human/rat dental stem cell transplantation protects RGCs and their axons from degeneration in rat optic nerve crush model<sup>36,37</sup>.</li> </ul>
Compressive	<ul style="list-style-type: none"> <li>Compressive optic neuropathy</li> </ul>	<ul style="list-style-type: none"> <li>Secondary to an orbital lesion</li> </ul>			
Infiltrative	<ul style="list-style-type: none"> <li>Inflammatory conditions (such as sarcoidosis), tumors, and infective agents</li> </ul>				
Glaucomatous	<ul style="list-style-type: none"> <li>Glaucoma</li> </ul>	<ul style="list-style-type: none"> <li>Chronic progressive and irreversible visual field defect</li> </ul>	<ul style="list-style-type: none"> <li>60.5 million people were affected by primary open-angle glaucoma and primary angle-closure glaucoma in 2010 and the number of people with glaucoma will increase to 111.8 million in 2040<sup>38</sup></li> </ul>	<ul style="list-style-type: none"> <li>Reduction of intraocular pressure is crucial, including drug therapy and surgery therapy<sup>39</sup></li> </ul>	<ul style="list-style-type: none"> <li>Intravitreal injection of rat bone marrow stromal cells shows less reduction in RGC numbers in chronic IOP elevation rat model<sup>40</sup>.</li> <li>Intravitreal injection of rat bone marrow-derived MSCs increases overall RGC axon survival and decreases the rate of RGC axon loss<sup>41</sup>.</li> <li>Intravitreal injection of human dental pulp stem cells or bone marrow-derived MSCs in rats increases RGC survival and protects RNFL thinning and RGC function<sup>42</sup>.</li> <li>Intravitreal injection of exosomes from bone marrow-derived MSCs promotes RGC protection and prevent RNFL thinning and retinal function loss<sup>43</sup>.</li> </ul>

HSC: hematopoietic stem cell; IOP: intraocular pressure; LHON: Leber hereditary optic neuropathy; MSC: mesenchymal stem cell; NMO: neuromyelitis optica; ON: optic neuritis; PDLSCs: periodontal ligament-derived stem cells; PION: posterior ischemic optic neuropathy; RGC: retinal ganglion cell; RNFL: retinal nerve fiber layer; TON: traumatic optic neuropathy.

are diverse, including vascular, inflammatory, compressive, infiltrative, traumatic, toxic, and nutritional optic neuropathies<sup>3</sup>. In contrast, hereditary optic neuropathies belong to those with underlying heritable etiology. Leber's hereditary optic neuropathy (LHON) and autosomal dominant optic atrophy (ADOA) are the most common hereditary optic neuropathies mostly affecting the children and teenagers<sup>45</sup>. Optic nerve damage in LHON and ADOA are usually observed in earlier life, and the optic nerve is permanently affected as the results of gene mutations<sup>46</sup>. Glaucoma is also considered as a complex genetic disease with the interplay between multiple associated genes and the environmental factors<sup>47</sup>. Glaucoma is characterized by the optic nerve head cupping and visual field defects<sup>47</sup>, and intraocular pressure (IOP) elevation is a reversible risk factor<sup>39</sup>.

In spite of different etiologies for different types of optic neuropathies, retinal ganglion cell (RGC) death and the axonal loss are the commonly shared pathophysiological changes for all types of optic neuropathies. RGCs collectively transmit the image-forming and non-image forming visual information from the retina to the lateral geniculate nucleus and superior colliculus in the brain; therefore, they are the important treatment target in plenty of the optic neuropathy research. RGC death in optic neuropathies is complex and complicated<sup>48</sup>, and RGC protection and regeneration are the major goals in the design of treatment regimes.

Clinically, a variety of medications and surgical procedures have been applied to different optic neuropathies in multiple clinical trials or routine practices, targeting the pathological conditions in different types of optic neuropathies. However, the visual defect of a significant number of patients with optic neuropathies still cannot be fully recovered under current clinical interventions. Completely rescuing RGCs from degeneration, activating their regeneration endogenously, and replenishing them exogenously are the major challenges for the clinicians and researchers. Besides, RGCs are vulnerable to stresses and injuries, and they have limited regenerative capacity. Partial functional recovery is possible; yet, the vision loss is inevitable and irreversible.

Basically, mammalian retina has low intrinsic regenerative capacity. Lack of trophic support, scar formation at the injury site, and the myelin-associated inhibitors further contribute to RGC degeneration and failure in axonal regeneration upon diseases and after injuries<sup>49</sup>. Many research studies strived through variable ways to alleviate RGC death and axonal loss, including supplying exogenous neurotrophic factors, electrical stimulation, inhibiting the apoptosis mediators, counteracting glial scar formation and the myelin-associated inhibitors, as well as gene and cell therapies. Exogenously supplying brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) can promote RGC survival in rodents after optic nerve injury<sup>50,36</sup>. Moreover, glial cell line-derived neurotrophic factor (GDNF), fibroblast growth factor (FGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), and insulin-like growth factors (IGFs) can also enhance RGC survival in

retinal degeneration models, such as ischemic/reperfusion model<sup>51</sup>. However, multiple injections of neurotrophic factors are needed to demonstrate a prominent effect of RGC protection. Trans-corneal electrical stimulation for 1 month shows increase in RGC survival with preservation of RGC function after hypertensive injury in mouse retina<sup>52</sup>. Inhibiting the apoptotic pathway by caspase-3 inhibitors, such as z-DEVD-fmk, has been reported to promote RGC survival<sup>53</sup>. By targeting the myelin-associated growth-inhibitory molecules, the inhibitor of myelin-associated glycoprotein can promote neurite outgrowth from dorsal root ganglia neurons<sup>54</sup>. Gene therapy mediated through the adeno-associated viruses (AAVs) on different target genes, such as ectopic expression of BDNF<sup>55</sup> and CNTF as well as knockdown of RhoA<sup>50</sup>, can enhance RGC survival and axonal regeneration in rodents. The disease types, tissue tropism of AAVs, the gene targets, the vector carrying capacity, potential genome integration, immune responses, as well as the treatment safety and toxicity should reasonably be considered before the clinical application of gene therapy<sup>56</sup>. It is also warranted to develop novel and effective treatments for optic neuropathies. Recently, stem cell therapy was proposed as a potential treatment for optic neuropathies. Adult stem cells, including mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs), have been applied in clinical trials based on their neuroprotective properties. In this article, the applications of adult stem cells on different types of optic neuropathies and the related mechanisms will be reviewed. Research updates on the strategies to enhance the neuroprotective effects of human adult stem cells will be summarized. In addition, the potential of cell replacement therapy by adult stem cells for optic neuropathies will also be discussed.

## An Overview and the Neuroprotective Effects of Adult Stem Cells

Stem cells are defined as the immature cells with the capability of self-renewal and differentiation into mature cells. They are essential for tissue growth, development, and homeostasis. According to the developmental stages, stem cells can be sub-classified into embryonic stem cells (ESCs) and adult stem cells. ESCs, derived from the inner cell mass of a blastocyst prior to gastrulation, are regarded as the gold standard of pluripotent stem cells, which are capable to differentiate into all cells of the three germ layers: ectoderm, mesoderm, and endoderm. Adult stem cells, referring to the stem cells originated from fully developed tissues, have been identified in multiple organs and tissues, including the umbilical cord, bone marrow, teeth, adipose tissue, brain, eye, intestine, and salivary glands<sup>57</sup>. Adult stem cells function to maintain the specificity of adult tissues through homeostatic cell replacement and tissue regeneration<sup>58</sup>. Although they are presumed quiescent within the adult tissues, they can divide infrequently to produce a stem cell clone and a transiently amplifying cell. The transiently amplifying cells can undergo

limited cell divisions before terminally differentiating into mature functional tissue cells. Adult stem cell differentiation is lineage restricted, and different types of adult stem cells have their specific functions in different tissues and organs. This review article focused on the research of HSCs, MSCs, and retinal stem cells in the treatment of optic neuropathies.

Hematopoietic stem cells (HSCs) can differentiate into all cell lineages in mature blood (erythroid, myeloid, and lymphoid) and sustain the blood production for the entire human life<sup>59</sup>. HSCs for transplantation in many blood-related diseases are mainly obtained from adult bone marrow, umbilical cord blood, and mobilized peripheral blood. HSCs can be sorted with the positive selection by CD34, CD45, and CD133 as well as the negative selection by CD31, CD105, and CD146<sup>60</sup>. Activation of HSCs is driven by the PI3K/AKT/mTORC1 signaling pathway, whereas their re-entry into quiescence depends on the MEK/ERK signaling pathway<sup>61</sup>.

Mesenchymal stem cells (MSCs) are the multipotent stromal cells with the capability differentiating into mesodermal lineages, including adipocytes, chondrocytes, and osteocytes<sup>62</sup>. Although MSCs were originally identified in bone marrow, they can also be found in different tissues, including adipose tissue, umbilical cord, and teeth<sup>63</sup>. Human MSCs can be sorted with the positive selection by CD29, CD44, CD73, CD90, CD105, CD146, and STRO-1 as well as the negative selection by CD31, CD34, CD45, CD49f, and CD133<sup>64</sup>. Apart from the expression of specific cell surface markers, MSCs are also defined to be growing in adherence to the plastic surface while maintained in standard culture conditions and are capable to be *in vitro* induced into mesenchymal lineages with appropriate medium as recommended by the International Society of Cellular Therapy<sup>65</sup>.

Retinal stem or progenitor cells (RPCs) are the neural stem cells generating all seven types of mature retinal cells: cone/rod photoreceptors, bipolar cells, horizontal cells, amacrine cells, RGCs, and Müller glia. RPCs are originated from neuroectoderm during development<sup>66</sup> and have been reported to be resided in the retina and the pigmented ciliary margin in zebrafish, chick, and rodent<sup>67</sup>. Moreover, Müller glia and retinal pigment epithelium have also been suggested demonstrating the RPC properties through de-differentiation upon injury<sup>68</sup>. The expressions of PAX6, VSX2, RAX, SOX2, Nestin, and c-Kit can be found in RPCs, but negative for SSEA4<sup>69</sup>.

Endogenous regeneration by stem cells upon diseases or after injuries in human retina, referring to the progenitor cells localized in the ciliary margin zone of the retina and the Müller glia, is very rare and largely unknown. Besides, the collection of RPCs from a living patient could possibly further damage the retina, which is not ethical. Therefore, treatments using adult stem cells for optic neuropathies mainly rely on the application of autologous/heterologous stem cells from other tissues, including HSCs and MSCs.

Adult stem cells, in addition to the capability differentiating into lineage-specific mature cells, also possess the

neuroprotective properties, especially for HSCs and MSCs. The neuroprotective effect is mainly mediated by the paracrine bystander effect, including modulating the plasticity of the host damaged tissues, secreting the survival-promoting and neurotrophic factors, restoring the release of synaptic transmitters, integrating into the existing neural and synaptic networks, and rebuilding the afferent and efferent connections<sup>70</sup>. Moreover, MSCs also demonstrate the immunomodulatory properties<sup>71</sup>, allowing autologous/heterologous transplantation without additional immunosuppression treatment. MSCs have been found to facilitate cell repair via immunomodulation<sup>72,73</sup>. In addition, organelle or material transfer, such as mitochondrial transfer, from MSCs into the targeted cells through tunneling nanotubes can also rescue cells from damage and apoptosis<sup>74,75</sup>. Importantly, the neuroprotective properties are naturally acquired by adult stem cells without the need of reprogramming or genetic modification, and the usage of adult stem cells has no moral objection or ethical controversies. The risk of teratoma formation is very limited after adult stem cell transplantation<sup>76</sup>. Despite the accessibility and naturality of adult stem cells, the retina is comparatively resistant to transplant integration with limited long-term xenograft survival. Application of erythropoietin or chondroitinase ABC can facilitate the transplanted cell migration into the retina<sup>77</sup>. Adult stem cell treatment should be promising for optic neuropathies.

## Application of Adult Stem Cells for Different Types of Optic Neuropathies

### Application of Adult Stem Cells for Demyelinating Optic Neuritis

Demyelinating ON is one of the most common causes of unilateral or bilateral painful visual loss in young adults<sup>4</sup>. The incidence of ON was estimated as five per hundred thousand individuals annually, with a prevalence of 115 per hundred thousand individuals in the United States<sup>8</sup>. Demyelinating ON can be an early sign of neuromyelitis optica spectrum disorder (NMOSD), multiple sclerosis, or other autoimmune disorders on the spinal cord, brain, and optic nerve<sup>4</sup>. The anti-aquaporin-4 (AQP4) and anti-myelin oligodendrocyte glycoprotein (MOG) auto-antibodies are discovered in some demyelinating ON patients, but targeting different tissues under different pathogenesis<sup>5,6</sup>. The pathophysiological mechanism of AQP4 antibody-related ON could be related to the binding of AQP4 antibody (AQP4-IgG or AQP4-Ab) to the AQP4 water channel on the surface of astrocytes, resulting in astrocyte cytotoxicity along with secondary oligodendrocyte damage and demyelination<sup>5</sup>. On the other hand, the pathophysiological mechanism of the MOG antibody-related ON is caused by the antibodies directed against MOG on the oligodendrocytes<sup>6</sup>. There is also a group of ON patients without identification of specific auto-antibody. However, no targeted treatment



for demyelinating ON is clinically available. High dose of intravenous methylprednisolone (IVMP), aiming to reduce the inflammation at the optic nerve, can promote visual function improvement in the patients with ON<sup>78</sup>. In addition, the plasma exchange treatments, oral steroids, and immunosuppressive and immunomodulatory drugs can also be applied to prevent further relapse<sup>79</sup>. Nevertheless, high dose IVMP and the enhanced immunosuppressive treatment will extensively suppress the normal immune function, leading to severe side effects and increasing the risk of serious infection. Application of adult stem cells, especially MSCs, in ON should help with the immunomodulation, anti-inflammation, and enhancing RGC survival<sup>18,80,81</sup>.

In the experimental MOG-induced autoimmune encephalomyelitis model resembling multiple sclerosis, tail vein injection of human bone marrow-derived MSCs has been shown to alleviate RNFL thinning and RGC death as well as protect the pattern electroretinography response with reducing the HIF-1 signaling activation and upregulating the Abca1 expression<sup>80</sup>. For the clinical study of MSC therapy on ON and NMOSD (Table 2), the clinical trial (NCT02249676) on 12 neuromyelitis optica (NMO) patients, one recurrent ON patient, and two patients with recurrent longitudinally extensive transverse myelitis received autologous MSC transplantation, showed that increase in RNFL thickness and optic nerve diameters with visual function improvement could be observed at 1 year after transplantation<sup>18</sup>. Another clinical trial (NCT01920867) on a 54-year-old female subject with autoimmune ON reported improvements in visual acuity, visual field, and RNFL thickness after autologous bone marrow-derived MSC transplantation<sup>19</sup>. Moreover, five Chinese NMO patients treated with human umbilical cord-derived MSCs showed improvement in symptoms, and the signs were improved in four study subjects with reduction in relapse frequencies, magnetic resonance imaging (MRI) characteristics, and the severity of lesions<sup>15</sup>. Although few adverse events reported, peripheral blood B lymphocytes were inhibited, and T lymphocytes increased after treatment<sup>15</sup>. In addition, a clinical trial (NCT01920867) on a 27-year-old female subject with idiopathic bilateral ON reported that the visual acuity of the eyes receiving retrobulbar, subtenon, and intravitreal stem cell injections improved from 20/800 to 20/100 while those receiving intra-optic nerve injection of stem cells and vitrectomy improved from 20/4,000 to 20/40 at 6 months after autologous bone marrow-derived MSC injection<sup>17</sup>. The visual acuity improvement remained stable even after the 12-month follow-up period. However, the transplanted bone marrow-derived MSCs in patients with NMO exhibit the decreased proliferation capacity and increased cell death rate<sup>82</sup>. Furthermore, there are 2 clinical trials applying the autologous bone marrow-derived MSCs, including the SCOTS2 (NCT03011541) in the United States for the NMO treatment, and the Treatment of Optic Neuropathies Using Autologous Bone Marrow-Derived Stem Cells (NCT02638714) in Jordan for the safety and efficacy on functional restoration in ON.

For the clinical study on HSCs, a study on 13 NMOSD patients with the infusion of mobilized autologous peripheral blood stem cells, one patient with coexistent systemic lupus erythematosus died due to the complications of active lupus; yet, the mental (from 37.6 to 65), physical (from 28 to 56), and total quality of life (from 34.2 to 62) were improved in 11 patients after a 5-year follow-up period<sup>16</sup>. Critically, among the 11 patients with sero-positive on baseline AQP4-IgG, nine patients became sero-negative after stem cell transplantation. Moreover, in a 23-year-old Chinese female NMO subject who received autologous peripheral HSC transplantation, the visual acuity of the right eye increased from 0.02 to 0.04 at 6 months after transplantation<sup>14</sup>. In spite of the remaining atrophic optic nerve, other symptoms were improved, including the muscle power. In contrast, a study on 21 opticospinal MS patients with intravenous transplantation of autologous peripheral blood stem cells reported no significant improvement after transplantation even though the expanded disability status scale score was decreased<sup>83</sup>. Besides, failure in preventing the relapse of NMO was also reported in one patient with autologous HSC transplantation, which could be due to the inadequate reset of immune tolerance or sustained autoimmunity to aquaporin-4 auto-antigen<sup>84</sup>. There are also two clinical trials on autologous HSCs for NMO withdrawn or terminated without any data released: The Autologous Transplant To End NMO Spectrum Disorder (ATTEND; NCT03829566) in the United States and the Autologous Hematopoietic Stem Cell Transplant in Neuromyelitis Optica (SCT-NMO; NCT01339455) in Canada.

### *Application of Adult Stem Cells for Non-Arteritic Anterior Ischemic Optic Neuropathy*

Non-arteritic anterior ischemic optic neuropathy (NAION) belongs to one of the most common acute optic neuropathies with an incidence of 2.3 to 10.3 per hundred thousand individuals per year in the United States<sup>21</sup>. The pathophysiology of NAION is presumed as the acute hypoperfusion of short posterior ciliary arteries, resulting in insufficient blood flow to the optic nerve head and anterior optic nerve segment<sup>85,86</sup>. The ischemic insult becomes worsen by the optic disc edema compressing the axon and capillaries and leads to a compartment syndrome<sup>86</sup>. Along with the ischemia, the cytotoxic factor and cytokine release would lead to further damages on the optic nerve<sup>87</sup>, and eventually, optic nerve atrophy would develop<sup>86</sup>. Different therapeutic strategies for NAION have been investigated, including intravitreal, peri-ocular, systemic, and surgical therapies<sup>88</sup>, to reduce the related vascular risk factors, improve the perfusion of the optic nerve by vasodilators, relieve the optic nerve head pressure, inhibit cytotoxic factors by the molecular targeted therapy, and promote optic nerve axonal regeneration. Application of adult stem cells in NAION should be able to improve the vascular circulation and perfusion and nourish the RGCs with the neurotrophic factors.

**Table 2.** Registered Clinical Trials of Adult Stem Cell Therapy for Optic Neuropathies.

Identifier	Country	Status	Study	Phase	Estimated number of patients	Estimated trial end	Targeted diseases
NCT01920867	United States	Enrolling by invitation	Stem Cell Ophthalmology Treatment Study (SCOTS)	Not applicable	300	2020	Retinal disease Macular degeneration Hereditary retinal dystrophy Optic nerve disease Glaucoma
NCT00278486	United States	Terminated	Hematopoietic Stem Cell Transplantation in Autoimmune-Related Retinopathy (ARRON)	Phase I	2	2012	Retinal disease
NCT02144103	Russia	Unknown	Effectiveness and Safety of Adipose-Derived Regenerative Cells for Treatment of Glaucomatous Neurodegeneration	Phase I/II	16	2019	Retinal degeneration
NCT01364246	China	Unknown	Safety and Efficacy of Umbilical Cord Mesenchymal Stem Cell Therapy for Patients With Progressive Multiple Sclerosis and Neuro-myelitis Optica	Phase I/II	20	2014	Primary open-angle glaucoma Progressive multiple sclerosis neuromyelitis optica
NCT02330978	Brazil	Completed	Intravitreal Mesenchymal Stem Cell Transplantation in Advanced Glaucoma	Phase I	10	2016	Primary open-angle glaucoma
NCT02638714	Jordan	Recruiting	Treatment of Optic Neuropathies Using Autologous Bone Marrow-Derived Stem Cells	Phase I/II	100	2021	Optic neuropathy
NCT03011541	United States	Recruiting	Stem Cell Ophthalmology Treatment Study II (SCOTS2)	Not Applicable	500	2021	Optic neuropathy
NCT01834079	India	Unknown	Study the Safety and Efficacy of Bone Marrow-Derived Autologous Cells for the Treatment of Optic Nerve Disease (OND)	Phase I/II	24	2016	Optic Atrophy
NCT03173638	Spain	Recruiting	Safety Assessment of Intravitreal Mesenchymal Stem Cells for Acute Non-Arteritic Anterior Ischemic Optic Neuropathy	Phase II	5	2021	Non-Arteritic ischemic optic neuropathy
NCT03829566	United States	Withdrawn	Autologous Transplant to End NMO Spectrum Disorder (ATTEND)	Phase II/III	50	2025	Neuromyelitis optica Devic's disease
NCT01339455	Canada	Terminated	Autologous Hematopoietic Stem Cell Transplant in Neuromyelitis Optica (SCT-NMO)	Phase I/II	3	2018	NMO spectrum disorder Neuromyelitis optica
NCT02249676	China	Completed	Autologous Mesenchymal Stem Cells for the Treatment of Neuromyelitis Optica Spectrum Disorders	Phase II	15	2014	Devic's syndrome Devic's neuromyelitis optica Devic's syndrome Devic's disease Devic's disease
NCT00787722	United States	Completed	Hematopoietic Stem Cell Transplant in Devic's Disease	Phase I/II	13	2018	Devic's disease
NCT02976441	United States	Withdrawn	Autologous Stem Cell Collection and Reinfusion in Newly Diagnosed High-Grade Gliomas	Early Phase I	0	2018	Astrocytoma Brainstem glioma Ependymoma Mixed glioma Oligodendroglioma Optic nerve glioma

Information obtained from <http://clinicaltrials.gov/>. NMO: neuromyelitis optica.

In a mouse model of NAION, intravenous ( $1 \times 10^6$  cells in 0.2 ml) or intravitreal injection ( $2 \times 10^5$  cells in 3  $\mu$ l) of adult mouse bone marrow–derived stem cells at 1 month showed stem cell migration to various retinal layers and differentiation into endothelium, immune cells, astrocytes, and neural cells, indicating that the transplanted adult bone marrow cells can contribute to the retinal remodeling after ischemia by promoting cell regeneration and sustaining endogenous repair<sup>89</sup>. Early neural and sustained astrocyte differentiation from adult bone marrow–derived stem cells could be regulated through the Flt-1-mediated vascular endothelial growth factor (VEGF) signaling<sup>89</sup>. In addition, both BDNF and CNTF can enhance the neural differentiation of adult bone marrow–derived stem cells in the retina of AION mice<sup>90</sup>. In a rat model of AION, intravitreal injection of human Wharton's jelly MSC-derived conditional medium can inhibit RGC apoptosis and microglia activation and preserve visual function<sup>81</sup>. Notably, the transplantation of human Wharton's jelly MSCs in normal rats would cause severe inflammation and impair the retinal structure and function as compared with the phosphate buffered saline (PBS)-treated rats<sup>81</sup>. The effect of xenotransplantation of human MSCs cannot be ignored.

Currently, there are two clinical studies using adult stem cells for NAION (Table 2). The Stem Cell Ophthalmology Treatment Study (SCOTS; NCT01920867), conducted in the United States, is a non-randomized study based on the combinations (retrobulbar, subtenon, intravitreal, and intravenous) of autologous bone marrow–derived stem cell injection. In SCOTS, 10 NAION patients received autologous bone marrow–derived stem cell treatment<sup>91</sup>. About 80% patients showed improvement in Snellen binocular vision within 6 months, and 20% remained stable. This study showed an average of 22.74% vision improvement in Snellen and maximum 83.3% improvement in logMAR visual acuity, with an average logMAR visual acuity gain of 0.364 in the treated eyes.

Another Phase II clinical trial for acute NAION in Spain (NCT03173638) aimed to assess the safety and inflammatory reaction of intravitreal allogenic bone marrow–derived MSC injection in the acute phase of NAION based on the neuroprotective properties of MSCs to reduce the progressive axonal degeneration in NAION. The outcome of the study has not been reported yet.

### ***Application of Adult Stem Cells for Traumatic Optic Neuropathy***

Traumatic optic neuropathy (TON) refers to the optic nerve injured directly or indirectly or by a blast. In 1998, the World Health Organization estimated trauma causing 1.6 million binocular blindness, 2.3 million people with both eyes low vision, and 19 million monocular blindness<sup>92</sup>. The injuries from traffic accidents, sports, and recreational activities account for 78.2% cases in India<sup>93</sup>. Direct optic nerve injury

includes penetrating trauma and impinging injury by bone fragments or orbital hemorrhage. Indirect injury commonly occurs during head or globe injury when the optic nerve could be sheared and avulsed by the rotational forces<sup>94</sup>. The energy can be transmitted through the skull to the optic nerve and retina, causing rapid changes in IOP and inducing RGC damage<sup>94</sup>. Following the direct mechanical disruption on the axons of the optic nerve, the enzymatic processes could be activated to degrade the cytoskeleton, leading to the delayed impairment of the axonal structure and function<sup>95</sup>. In the TON treatment trial (TONTT) study, daily intravenous administration of erythropoietin (stimulating erythrocyte production) or methylprednisolone for three consecutive days significantly improves the best corrected visual acuity and color vision in the TON patients<sup>94</sup>. Adult stem cells, with the secretion of neurotrophic factors and exosomes, can nourish the microenvironment in TON so as to promote RGC survival and axonal regeneration.

To mimic the clinical situation of TON, the experimental model of optic nerve injury by crushing the optic nerve in rodents is adopted. Intravitreal injection of rat bone marrow–derived stem cells ( $5 \times 10^6$  cells in 5  $\mu$ l) can increase RGC survival by 1.6 folds and promote axonal regeneration at 2 weeks after injection as compared with those with saline or dead cell injection<sup>35</sup>. Similarly, our group demonstrated that intravitreal injection of human periodontal ligament–derived stem cells (PDLSCs) can enhance RGC survival and axonal regeneration after optic nerve injury in rats, possibly through direct cell–cell interaction as well as neurotrophic factor secretion by PDLSCs<sup>36</sup>. Higher expression of FGF-2 and IL-1 $\beta$  in the transplanted retina can explain the neuroprotective effect of MSCs<sup>96</sup>. Human or rat dental stem cells can secrete nerve growth factor (NGF) and BDNF to promote RGC survival and axonal regeneration in the rat optic nerve crush model through the Trk receptor<sup>36,37</sup>. Our group also found that retinal injury can enhance the secretion of BDNF from human PDLSCs<sup>36</sup>. In addition, intravitreal injection of rat MSCs and human Wharton's jelly MSCs as well as its extracellular vesicles can enhance axonal regeneration and synaptic reconnection in superior colliculus, but fail to preserve the visual behavior in rodents with optic nerve injury<sup>97</sup>, indicating that MSCs can promote long-distance axonal regeneration. Yet, the neuroprotective mechanisms of MSCs still require further investigations. Besides, there is still no clinical trial on human stem cells for TON.

### ***Application of Adult Stem Cells for Leber's Hereditary Optic Neuropathy***

LHON belongs to one of the mitochondrial disorders with an incidence of 1 per 100,000 individuals<sup>45</sup>. LHON is maternally inherited due to the point mutations in mitochondrial DNA, leading to the reduction in mitochondrial ATP production and the elevation in oxygen-free radicals. The oxidative



stress in turn leads to the dysfunction and apoptosis of RGCs<sup>98</sup>. For the treatment, creatine and dietary antioxidant supplements, including coenzyme Q10 and L-carnitine, have been suggested to enhance mitochondrial bioenergetics and to ameliorate the neurodegenerative process<sup>99</sup>. Application of adult stem cells in LHON might be able to transfer healthy mitochondria to the diseased RGCs through cell–cell contact-mediated cellular material transfer so as to enhance RGC survival.

LHON mouse model has been established<sup>100</sup>; yet, no stem cell transplantation study has been tested in this mouse model. Alternatively, patient-specific LHON model can also be generated from induced pluripotent stem cells (iPSCs)<sup>101</sup>. Repairing mitochondrial dysfunction via mitophagy activation could be a possible treatment strategy for LHON<sup>102</sup>. For the LHON clinical trial on human adult stem cells (Table 2), SCOTS (NCT01920867) recruited five LHON patients, who received the transplantation of autologous bone marrow-derived stem cells<sup>29</sup>. The visual acuity and peripheral vision persistently increased, improving from finger counting to 20/100 and hand motion to 20/200. However, the thicknesses of macula and optic nerve head are not correlated with the vision improvement. Although adverse or serious adverse event was not found in this study, the sample size was relatively too small to draw the conclusion.

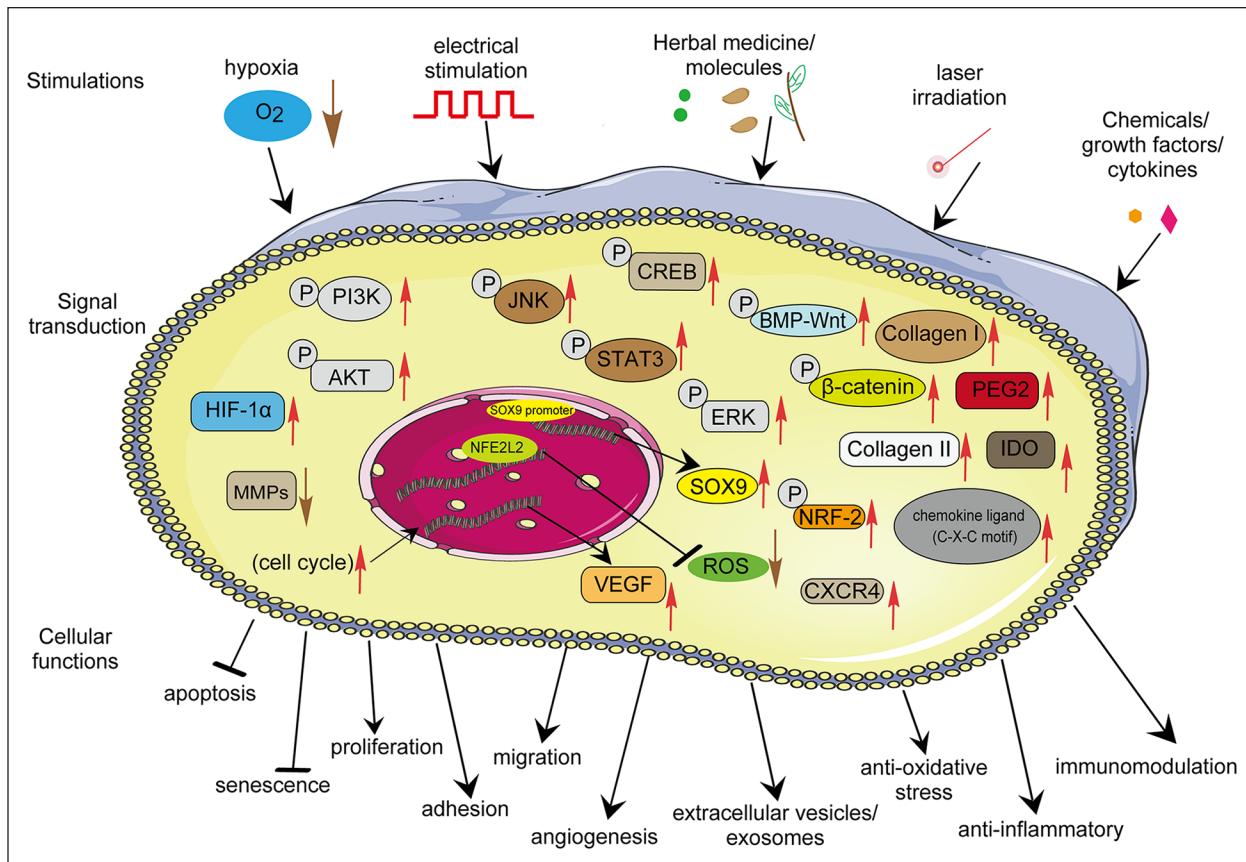
### *Application of Adult Stem Cells for Glaucoma*

Glaucoma, characterized by the optic disc cupping and progressive RGC degeneration<sup>39</sup>, is a leading cause of irreversible visual impairment and blindness in the world. It was estimated that 60.5 million individuals suffered from glaucoma in 2010, and the glaucoma patients will increase to 111.8 million in 2040<sup>38</sup>. The risk factors include aging, gender, ethnicity, hypertension, smoking, myopia, and diabetes<sup>103</sup>. Lamina cribrosa is considered as the primary site of injury. The imbalanced IOP level imposes mechanical stress to RGCs and leads to RGC death<sup>104</sup>. Although the pathogenesis of glaucoma is yet to be elucidated, various theories have been proposed, including the vascular, biochemical, and biomechanical theories<sup>104</sup>. For the biomechanics, the elevated IOP, together with the concept of the decreased intracranial pressure and the high trans-lamina cribrosa pressure difference, induces lamina cribrosa pore distortion, nerve fibers compression, and RGC death<sup>105</sup>. For the vascular theory, the decreased ocular perfusion caused by the elevated IOP can lead to intraneural ischemia, which would lead to RGC damage owing to inadequate supply of oxygen and nutrients as well as the activation of astrocytes and Müller glia. The elevated endothelin-1 and deranged nitric oxide-cyclic GMP signaling could also be involved in the vascular dysregulation<sup>106</sup>. The biochemical theory suggests that the involvement of reactive oxygen species (ROS), excitatory amino acids, nitric oxide, caspases, matrix metalloproteinases (MMPs), and tumor necrosis factor- $\alpha$  could lead to

RGC apoptosis and neuroinflammation in glaucomatous optic neuropathy<sup>107,108</sup>. Current clinical treatments mainly focus on lowering the IOP, and they can effectively delay the disease progression in majority of the glaucoma patients. The development of IOP-independent strategies to enhance RGC survival is warranted<sup>108</sup>. Application of adult stem cells in glaucoma should be able to improve the vascular circulation for better nutrient supply, exert the anti-inflammatory and anti-oxidative effects, and enhance RGC survival by the paracrine effect of adult stem cells.

In the chronic IOP elevation rat model by ligating the episcleral veins, RGC reduction was ameliorated in rats with intravitreal injection of rat bone marrow stromal cells ( $4 \times 10^4$  cells in  $5 \mu\text{l}$ )<sup>40</sup>. The transplanted bone marrow stromal cells were present along the inner limiting membrane or integrated into the nerve fiber and GC layer, but rarely expressed the neural lineage markers. Increased expression of bFGF and CNTF in bone marrow stromal cell treatment could explain the RGC protective effect. In the laser-induced IOP elevation rat model, intravitreal injection of rat bone marrow-derived MSCs ( $5 \times 10^6$  cells in  $5 \mu\text{l}$ ) increases RGC axonal survival and decreases axonal loss at 4 weeks after transplantation<sup>41</sup>. Comparatively, intravenous transplantation of bone marrow-derived MSCs showed no effect on optic nerve damage rescue. Moreover, intravitreal injection of human bone marrow-derived MSCs or dental pulp stem cells ( $1.5 \times 10^5$  cells in  $5 \mu\text{l}$ ) in rats before bi-weekly intracameral injection of TGF- $\beta$ 1 can increase RGC survival and protect retinal nerve fiber layer thinning and RGC function at 1 month after transplantation, which was not observed in adipose-derived stem cells<sup>42</sup>. Mouse bone marrow-derived MSC-promoted RGC survival in acute IOP elevation mice model could be mediated through the miR-21/PDCD4 axis with the suppression of caspase-8-mediated apoptosis, microglia activation, and inflammatory mediator production<sup>109</sup>. Apart from stem cell transplantation, intravitreal injection of small extracellular vesicles collected from bone marrow-derived MSCs can also promote RGC protection and prevent retinal nerve fiber layer degenerative thinning and the retinal function loss in the microbeads and laser photocoagulation-induced IOP elevation rat model<sup>43</sup>. The RGC protection mechanism of MSC-derived small extracellular vesicles could be mediated through miRNAs that, with the knockdown of Argonaute-2, the protective effects of MSC-derived exosomes on RGC soma and axons after optic nerve injury would be diminished<sup>110</sup>.

Up to now, there are four clinical trials studying adult stem cells in glaucoma (Table 2): SCOTS (NCT01920867) and SCOTS2 (NCT03011541) in the United States on autologous bone marrow-derived stem cells; the Effectiveness and Safety of Adipose-Derived Regenerative Cells for Treatment of Glaucomatous Neurodegeneration (NCT02144103) in Russia on subtenon injection of autologous adipose-derived regenerative cells for primary open-angle glaucoma; and the Intravitreal Mesenchymal Stem Cell



**Figure 1.** Enhancement of adult stem cell properties. Priming or preconditioning of adult stem cells can enhance the properties of adult stem cells with hypoxia, electrical stimulation, laser irradiation or by chemicals, growth factors, cytokines or herbal medicine/molecules. CREB: cAMP response element-binding protein; CXCR: chemokine signal-regulated kinase; HIF: hypoxia inducible factor; IDO: indoleamine 2,3-dioxygenase; JNK: c-Jun N-terminal kinase; MMPs: matrix metalloproteinases; NRF-2: nuclear factor erythroid 2-related factor 2; PEG2: prostaglandin E2; ROS: reactive oxygen species; SOX9: sex determining region Y-box transcription factor 9; STAT3: signal transducer of activators of transcription 3.

Transplantation in Advanced Glaucoma (NCT02330978) in Brazil on intravitreal autologous bone marrow-derived MSC transplantation for the worst eye of legal bilaterally blinded glaucoma patients. The two advanced glaucoma patients in NCT02330978 trial did not show improvements on visual acuity, visual field, and electroretinographic (ERG) responses after transplantation. Yet, retinal detachment with proliferative vitreoretinopathy was found in one patient at 15 days after MSC transplantation<sup>111</sup>. The strategy of MSC transplantation in glaucoma patients should be a concern, and modifications may be needed. The safety of intravitreal MSC transplantation requires further investigations. The results of other three clinical trials have not been released yet.

### Enhancement of the Neuroprotective Effects for Adult Stem Cell Application

Although adult stem cell transplantation has been proven to alleviate RGC death and promote axonal regeneration in different RGC degeneration models, the neuroprotective effect

of adult stem cells is still modest. The regenerative properties can be influenced by the intrinsic and extrinsic factors, such as age effect<sup>112</sup> and cigarette smoke component<sup>113</sup>. To boost up the treatment effect of adult stem cells for optic neuropathies, priming or preconditioning of adult stem cells is a prospective strategy to enhance adult stem cell properties before transplantation. These include improving the grafting ability of adult stem cells, increasing stem cell survival in host tissues, and promoting the paracrine effects and migration ability of stem cells. Recently, there are growing research interests to explore different attempts to modulate the properties of adult stem cells (Fig. 1).

### Hypoxia

Hypoxic condition (2% oxygen) has been demonstrated to increase the proliferation rate and stemness of adipose-derived stem cells without altering cell morphology<sup>114</sup>, which promotes DNA synthesis and reduces the expression of MMPs<sup>114</sup>. Human bone marrow-derived stem cells and

umbilical blood CD133<sup>+</sup> cells, when exposed to 1.5% oxygen for 24 h, could increase total colony numbers with hypoxia inducible factor (HIF)-1 $\alpha$  upregulation<sup>115</sup>. The increase in stem cell survival after hypoxia treatment is associated with the expression of HIF-1 $\alpha$  through the increased phosphorylation of AKT and p38 MAPK<sup>116</sup>. Low oxygen-activated HIF-1 $\alpha$  delays cell senescence with macrophage inhibitory factor activation and p53-mediated pathway inhibition<sup>117</sup>. In addition, canine adipose tissue-derived MSCs preconditioned with deferoxamine (a hypoxia-mimetic agent) exhibit an increased secretion of anti-inflammatory prostaglandin E2 and tumor necrosis factor- $\alpha$ -stimulated gene-6, while decrease the M1 macrophage but increase the M2 macrophage marker expression<sup>118</sup>. Furthermore, hypoxic preconditioning prior to transplantation can suppress MSC apoptosis and increase the migration abilities with higher CXCR4 expression, resulting in promoting neuronal regeneration and improving neural function after transplantation in rat model of middle cerebral artery occlusion<sup>119</sup>.

### Chemicals, Growth Factors, and Cytokines

FGF can promote the proliferation of MSCs from different sources<sup>120,121</sup>. Preconditioning human umbilical cord blood-derived MSCs with both interferon- $\gamma$  (IFN- $\gamma$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) can induce regulatory T-lymphocyte differentiation and inhibit Th1 T-lymphocyte differentiation with increasing prostaglandin E2 secretion and indoleamine 2,3-dioxygenase (*IDO*) and cyclooxygenase 2 (*COX2*) gene expression<sup>122</sup>. Melatonin preconditioning can increase MMP-9 and MMP-13 expression in bone marrow-derived MSCs<sup>123</sup>, and improves transplanted bone marrow-derived MSC survival and engraftment, which promotes renal regeneration in chronic kidney disease rat model<sup>123</sup>. Moreover, preconditioning with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) also reported to enhance the proliferation, clonogenicity, adhesion, and migration of decidua basalis-derived MSCs and reduce IL-1 $\beta$  expression<sup>124</sup>. H<sub>2</sub>O<sub>2</sub> preconditioning can increase superoxide dismutase, NQO1, catalase, and heme oxygenase 1 expression in bone marrow-derived MSCs via nuclear factor erythroid 2-related factor 2 pathway, resulting in decrease in intracellular ROS levels, increase in the anti-oxidative stress ability, and promotion of bone marrow-derived MSC survival<sup>125</sup>. In addition, preconditioning human cardiac stem cells with cobalt-protoporphyrin (heme oxygenase-1 inducer) can result in greater improvement in left ventricular remodeling by protecting human cardiac stem cells from apoptosis via ERK/NRF-2 signaling pathway activation<sup>126</sup>. Thrombin preconditioning can accelerate human umbilical cord blood-derived MSC-derived extracellular vesicles biogenesis and enrich the cargo contents by protease-activated receptor-mediated signaling pathways<sup>127</sup>, whereas less degradation of exosomes could be achieved by kartogenin preconditioning

on bone marrow-derived MSCs<sup>128</sup>. Furthermore, treatment of rat MSCs with valproic acid and lithium can stimulate cell migration through the HDAC/CXCR4 and GSK-3 $\beta$ /MMP-9 pathways, respectively<sup>129</sup>.

### Herbal Medicine and Molecules

We recently demonstrated that curcumin treatment (5  $\mu$ M) on human bone marrow-derived MSCs can enhance MMP13-mediated osteogenic differentiation, reduce MMP1 expression, and upregulate immunomodulatory gene *IDO1* expression although it would inhibit MSC proliferation and migration in high concentration (10  $\mu$ M or above)<sup>130</sup>. Treatment of a Chinese medicinal herbal formula, Danggui Buxue Tang (a mixture containing *Astragali radix* and *Angelicae sinensis radix*), can increase the number of bone marrow stromal cells and promote cell adhesion and migration via focal adhesion and PI3K/Akt signaling pathway activation<sup>131</sup>. Moreover, treatment of Chinese medicinal herbal formula Du-Huo-Ji-Sheng-Tang and its active constituent *Ligusticum chuanxiong hort* can delay human MSC aging process by decreasing cell senescence and increasing its osteogenic activity<sup>132</sup>. Treatment of *Salvia miltiorrhiza Bunge* attenuates apoptosis and improves cell viability of MSCs, leading to the recovery of the infarcted brain region and behavior in rat model of middle cerebral artery occlusion<sup>133</sup>. In addition, treatment of icariin (the active component of *epimedium koreanum* or *epimedium berberidaceae maxim*) promotes the survival of adipose-derived MSCs against oxidative stress, reduces cell apoptosis, and attenuates intracellular ROS through the PI3K/Akt-STAT3 signaling pathway<sup>134</sup>. Treatment of cajanine, isolated from the extracts of pigeon pea (*Cajanus cajan L. Millsp.*), can promote bone marrow-derived MSC proliferation by activating the cell cycle signal transduction pathway and accelerating their osteogenic differentiation<sup>135</sup>. Similarly, treatment of polydatin (isolated from the bark of *Picea sitchensis* or *Polygonum cuspidatum*) can enhance the proliferation of bone marrow-derived MSCs and the alkaline phosphatase activity via the BMP-Wnt/ $\beta$ -catenin signaling pathways<sup>136</sup>.

### Laser Irradiation

Low-level laser irradiation (LLLI; 660 nm, 30 mW) with 1 J/cm<sup>2</sup> energy density can enhance human PDLSC proliferation<sup>137</sup>. Moreover, LLLI (660 nm, 70 mW) with the energy density of 4 J/cm<sup>2</sup> can improve the bone healing process of adipose-derived stem cells in rats with critical-sized calvarial defects<sup>138</sup>. Furthermore, the proliferation, adipogenic differentiation ability, and VEGF, TGF- $\beta$ , and platelet-derived growth factor (PDGF) secretion of adipose-derived stem cells can be enhanced by 4 J/cm<sup>2</sup> GaAlAs low-level red laser (650 nm, 523 mW)<sup>139</sup>.



### Electrical Stimulation

Porcine bone marrow–derived MSCs preconditioned with nanosecond pulsed electric fields (10ns at 20kV/cm and 100ns at 10 kV/cm) enhances the chondrogenic differentiation with an increase in matrix deposition through the activation of JNK/CREB-STAT3 signaling pathway, and transplantation of preconditioned MSCs also enhances cartilage regeneration in rats suffered from articular cartilage defects<sup>140</sup>. Moreover, *in vitro* electrical preconditioning of human neural progenitor cells with a conductive polymer scaffold by a +1 V to –1 V square wave at 1 kHz for 1 hour can enhance stroke recovery in rat model of distal middle cerebral artery occlusion through VEGF-A pathway<sup>141</sup>. In addition, epidural stimulation of the motor cortex by electrical current for 1 month in normal rats can increase the proliferation and migration of progenitor cells in the subventricular zone<sup>142</sup>. Furthermore, in the rat ischemic stroke model, electrical stimulation (100  $\mu$ A, 100 Hz) for 2 weeks after injection of rat bone marrow–derived MSCs ( $62.5 \times 10^6$  cells/ml) showed amelioration in neurological severity score and reduction in the infarction areas<sup>143</sup>. Longer migration distance and wider migration area are also observed for the transplanted bone marrow–derived MSCs with electrical stimulation.

### Replacement Therapy for Optic Neuropathies by Adult Stem Cells

Cell replacement therapy conceptually aims to generate RGCs from stem cells to replace the diseased RGCs so as to rescue the visual function loss. New RGCs could be produced from different stem cell sources. Majority of research studies currently focus on the generation of RGCs from pluripotent stem cells, such as ESCs and iPSCs. Nevertheless, adult stem cells have also shown possessing the ability differentiating into retinal/neuronal lineage. The patients' stem cell–derived RGCs can serve as an *in vitro* platform carrying the patients' genome to study human disease pathology and mechanism as well as for drug screening and development<sup>101</sup>.

### Retinal Differentiation of Induced Pluripotent Stem Cells

Pluripotent stem cells, including ESCs and iPSCs, are the most promising sources of stem cells for cell replacement therapies due to their powerful differentiation capacities. The ESC therapy must be allogenic and is still under ethical controversial. In contrast, iPSCs can be generated from patients' somatic cells with the ability to differentiate into the cells of three germ layers, including retinal cells<sup>144</sup>. Importantly, iPSCs can also be used to generate retinal organoids<sup>145</sup>. Not only from the normal subjects, RGCs can also be generated from the iPSCs of the patients with Leber hereditary optic neuropathy<sup>146</sup> and autosomal dominant optic atrophy<sup>147</sup>.

Understanding the disease pathology and mechanism by the patients' iPSC-derived RGCs could be one of the important strategies in future optic neuropathy research.

### Retinal Differentiation of Müller Glia

Retinal Müller glia are believed acting like RPCs when responding to the retinal injury<sup>148</sup>. Human Müller cell line (MIO-M1) demonstrated the photoreceptor differentiation ability through the regulation of canonical Wnt signaling *in vitro*<sup>149–151</sup>. Moreover, transplantation of human Müller cell line (MIO-M1;  $3 \times 10^4$  cells in 3  $\mu$ l) in the laser-induced chronic IOP elevation rat model showed the capability to differentiate toward a neuronal phenotype rather than glial at 2 weeks after transplantation<sup>77</sup>. Although previous studies reported that adult stem cells directly injected into the retina could not spontaneously differentiate into retinal cells in the transplanted animal eyes, recent findings suggest that adult stem cells could also stimulate Müller glia re-entering the cell cycle and dedifferentiating to the RPC stage *in vivo*. The transplanted HSCs have been shown to fuse with Müller glia in the mouse model of N-methyl-N-nitrosourea-induced progressive photoreceptor degeneration that the Wnt signaling pathway is activated in the hybrid cells and drives the dedifferentiated hybrid cells toward the intermediate photoreceptor progenitor fate<sup>150,152</sup>. Not only the transplanted cells, the endogenously mobilized bone marrow cells could also fuse with Müller glia in the retina through the SDF1/CXCR4 axis regulation in the N-methyl-D-aspartate-induced retinal damage mouse model, which the hybrid cells could further differentiate toward a neuronal fate, including RGCs and amacrine cells.<sup>153</sup> Critically, the hybrid cells can also promote the regeneration of other damaged retinal cells. Retinal damage is critical for the hybrid cell formation *in vivo*, and retinal Müller glia can spontaneously fuse with the transplanted or endogenously mobilized HSCs. The hybrid cells can proliferate and commit to differentiation toward the mature retinal cells through the activation of the signaling pathways<sup>150,152,153</sup>. This discovery makes an advancement in the *in vivo* cell replacement therapy for retinal damage. Yet, the conversion efficiency, regenerated cell survival, and functional integration and rescue still need further in-depth investigations.

### Retinal Differentiation of Mesenchymal Stem Cells

Retinal differentiation is not limited to stem cells from retinal lineage since adult stem cells can give rise to an entirely distinct lineage of mature cells out of its physiological differentiation process. MSCs, which normally differentiate into chondrocytes, adipocytes or osteoblasts, can be induced into neural cells<sup>62</sup>. Upon bone marrow transplantation, genetic markers from donor's hematopoietic cells could be found in



multiple tissues, including brain cortex and cerebellum<sup>154</sup>. Moreover, intravitreally injected adult rat hippocampus-derived neural stem cells ( $5 \times 10^5$  cells in  $5 \mu\text{l}$ ) in the transient retinal ischemia-reperfusion adult rat model can integrate into the host retina and express neuronal differentiation marker Map2ab at 4 weeks after transplantation<sup>155</sup>. In addition, sub-retinal transplantation of adult hippocampus-derived neural progenitor cells in transient retinal ischemia model could display the neuron-like morphologies with fibers extending to RNFL at 4 to 5 weeks after transplantation, but they rarely co-express the retinal phenotypic markers<sup>156</sup>. The plasticity of adult stem cells could be interpreted by five possible mechanisms: cell fusion, transdifferentiation, dedifferentiation, heterogeneous stem cell populations, and pluripotency<sup>58</sup>. Cell fusion postulates that stem cells fuse with the host cells and acquire a mature phenotype of the embedded tissue. Transdifferentiation refers to the direct lineage conversion with the activation of dormant differentiation program to alter cell lineage specificity, whereas dedifferentiation hypothesizes that tissue-specific stem cells dedifferentiate spontaneously into basal multipotent cell state and re-differentiate into another cell lineage. Heterogeneity in stem cell population indicates a mixed population of stem cells found within the tissue origin, which might explain some events of transdifferentiation and dedifferentiation. Pluripotency is believed that pluripotent stem cells exist in adult tissues as a rare sub-population within the stem cell niches, and these pluripotent stem cells have been found in bone marrow-derived MSCs, spermatogonial stem cells, and the remnants of migrating neural crest<sup>157</sup>. Unlike iPSC reprogramming, retinal/neuronal differentiation of adult stem cells can bypass the complicated dedifferentiation process so as to reduce the risk of teratoma formation by the undifferentiated iPSCs upon transplantation<sup>158</sup>. Once the retinal/neuronal differentiation protocols of adult stem cells could be optimized, the *in vitro* neuron production could overcome the difficulty to collect retinal/neural progenitor cells from the patients.

Retinal differentiation of MSCs from different sources has been reported (Table 3). Human umbilical cord blood-derived MSCs and amniotic epithelial stem cells have been shown to be induced into cone/rod photoreceptor-like cells by inhibition of microRNA (miRNA)-203<sup>149</sup>. Mouse bone marrow-derived MSCs treated with BDNF, NGF, and bFGF can also express the photoreceptor and neural markers after 14-day induction, which can be enhanced by supplementation of human recombinant Wnt1<sup>152</sup>. Apart from photoreceptors, retinal pigment epithelial (RPE)-like cells have been reported to be generated from human adipose-derived stem cells<sup>159</sup>, and bone marrow-derived stem cells<sup>160</sup>. Moreover, rat bone marrow-derived MSCs can also be directed to RGC-like cells by co-culturing with postnatal day 1-3 rat retinal cells<sup>161</sup>. Coherent to other studies, we previously showed that human PDLSCs can be induced into retinal lineage with the treatment of noggin, Dkk-1, IGF-1, and bFGF with photoreceptor marker expression and glutamate-evoked calcium response<sup>162</sup>. Based on the noggin, Dkk-1, IGF-1, and

bFGF treatment, with the addition of BDNF, CNTF, NGF, and Shh, can direct human PDLSCs to RGC lineage after 24-day induction with neuronal and RGC marker expression, synapse formation, the glutamate-evoked calcium response, and spontaneous electrical activities<sup>163</sup>. The neuronal differentiation of human PDLSCs could be mediated by miRNA-132<sup>164</sup>. In addition to PDLSCs, we demonstrated that human adipose-derived stem cells can also be directed to retinal cells, and Notch signaling activator JAG1 can enhance the expression of RGC and RPC markers<sup>165</sup>. Furthermore, we revealed that spermatogonial stem cells possess the capability to differentiate toward the RGC lineage upon the 3-dimensional organoid culture<sup>166</sup>. Notably, apart from the pluripotent spermatogonial stem cells, pluripotent stem cells with neural crest origin could be isolated and purified from human PDLSCs by connexin-43<sup>167</sup>. These pluripotent adult stem cells should improve the retinal cell differentiation from human adult stem cell sources. Therefore, transplantation of adult stem cell-derived retinal cells should be a manageable treatment against retinal diseases in future<sup>69</sup>.

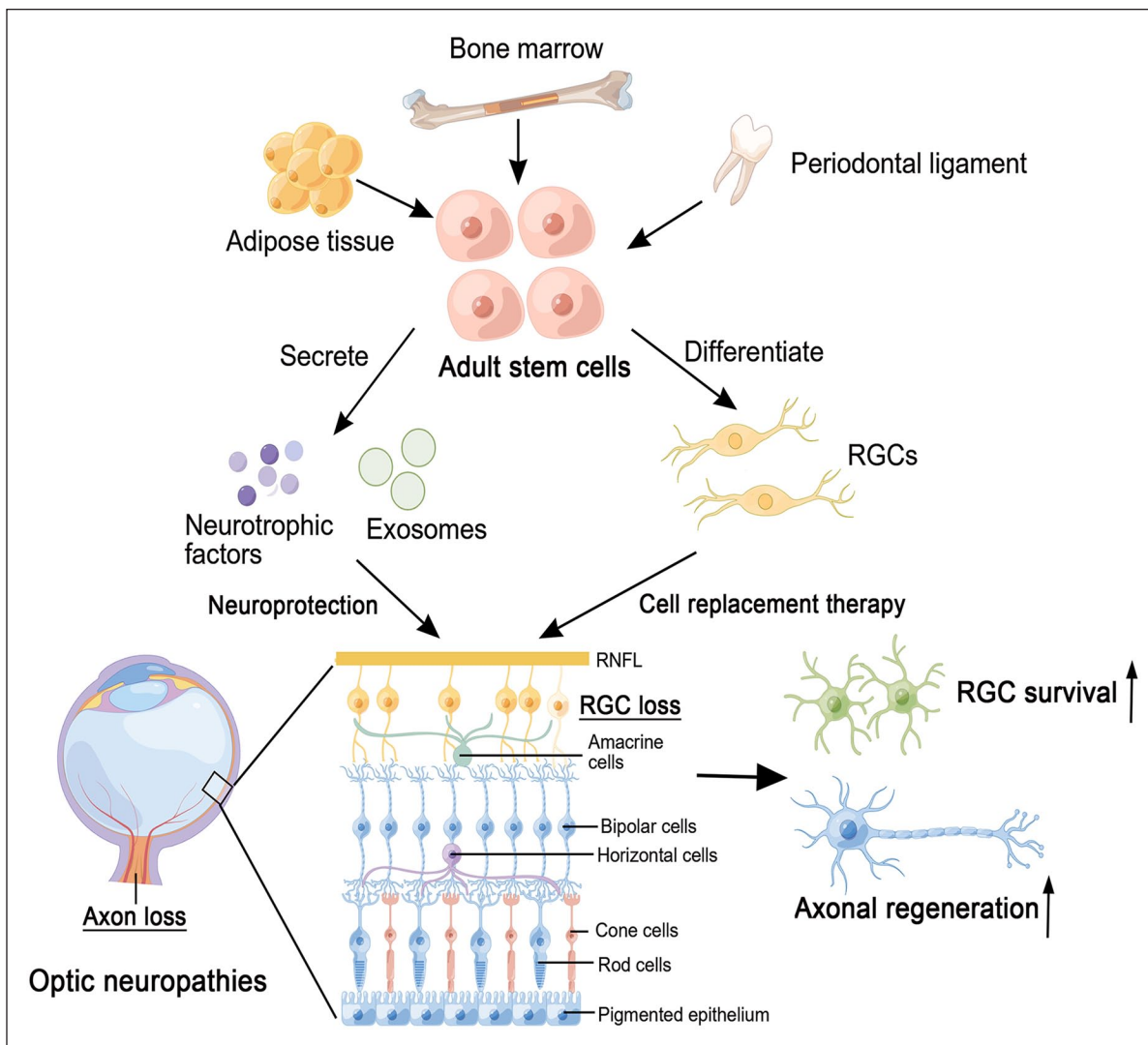
## Challenges and Prospects

The therapeutic potentials of adult stem cells on optic neuropathies have been intensively investigated (Fig. 2). The neuroprotective effect of HSCs and MSCs for RGC protection is convincing; yet, there are concerns and challenges for the adult stem cell treatments: (1) A standard guideline should be established to standardize the purity and condition of the transplanted adult stem cells as well as the modes of MSC transplantation since severe bilateral visual loss was reported in three age-related macular degeneration patients with intravitreal autologous adipose tissue-derived “stem cells” injections<sup>169</sup>, and retinal detachment with proliferative vitreoretinopathy was found in one patient at 15 days after MSC transplantation in a clinical trial study<sup>111</sup>. (2) Preconditioning could be a promising strategy to enhance the neuroprotective effects of adult stem cells; yet, studies in this area are still limited. Other preconditioning techniques and strategies as well as their mechanisms warrant further exploration and optimization. (3) Intravitreal transplantation of bone marrow-derived MSCs could induce reactive gliosis by activating the JAK/STAT3 and MAPK cascades in Müller glia, which could be a main obstacle for successful transplantation in RGC replacement and axonal regeneration<sup>170</sup>. (4) Stem cell-derived retinal cell integration and connection with the existing network in the host retina are the major challenges for the transplantation of exogenous stem cell-derived retinal cells. The connectivity of stem cell-derived RGCs could be hindered by the internal limiting membrane<sup>171</sup>. Activation of endogenous retinal stem cells or Müller glia to replace the diseased cells could be a possible alternative strategy to minimize these concerns<sup>172</sup>. (5) The majority of retinal disease modeling by stem cells currently relies on iPSCs<sup>101</sup>. Whether adult stem cells could similarly resemble the models of human diseases remains to be

**Table 3.** Retinal Differentiation of Adult Stem Cells.

Cell types	Species	Induction	Duration	Induced retinal cell type	Potential mechanisms
Müller cell line MIO-M1 <sup>150</sup>	Human	DMEM, 10% fetal calf serum, 20 ng/ml EGF2, 20 µM taurine, 5 µM retinoic acid, and 100 ng/ml IGF-1	7 days	Photoreceptor-like cells	Wnt signaling pathway
Bone marrow-derived stem cells <sup>160</sup>	Human	1. DMEM, 10% FBS, 0.1% rock inhibitor, 1% cell shield, inactivated human retinal pigment epithelium cells 2. DMEM, 10% FBS, inactivated human retinal pigment epithelium cells	1. 2 days 2. 5 days	Retinal pigment epithelium-like cells	/
Bone marrow-derived mesenchymal stem cells <sup>152</sup>	Mouse	DMEM/F-12 medium, 2% B27, 2% N2, 25 ng/ml BDNF, 40 ng/ml NGF, 25 ng/ml bFGF	14 days	Retinal neuron-like cells	Wnt/β-catenin signaling microRNA-410
Amniotic epithelial stem cells <sup>151</sup>	Human	Keratinocyte-Serum Free Medium, 0.031 µg/µl human recombinant EGF, 12.4 mg/ml bovine pituitary extract, 10% FBS, 30 nM anti-miRNA-410	21 days	Retinal pigment epithelium-like cells	microRNA-203
Amniotic epithelial stem cell <sup>149</sup>	Human	1. Keratinocyte-Serum Free Medium, 100 ng/ml Dkk-1, 10 ng/ml Noggin, 10 ng/ml IGF-1, 5 ng/ml bFGF SFM, anti-miRNA-203 2. DMEM/F12 medium, 1% B27, 1 ng/ml noggin, 1 ng/ml Dkk-1	21 days	Photoreceptor cells	/
Periodontal ligament-derived stem cells <sup>162</sup>	Human	DMEM/F12 medium, 1% B27, 10 ng/ml noggin, 10 ng/ml Dkk-1, N2	1. 3 days 2. 25 days	Retinal progenitor cells, photoreceptor	/
Periodontal ligament-derived stem cells <sup>163</sup>	Human	1. DMEM/F12 medium, 10% knockout serum replacement, 1x B27 supplement, 1 ng/ml noggin, 1 ng/ml Dkk-1, 5 ng/ml IGF-1 2. DMEM/F12 medium, 1x B27 supplement, 1x N2 supplement, 100 ng/ml noggin, 10 ng/ml Dkk-1, 100 ng/ml bFGF 3. DMEM/F12 medium, 1x B27 supplement, 1x ITS supplement, 10 ng/ml noggin, 10 ng/ml Dkk-1, 10 ng/ml IGF-1, 50 ng/ml bFGF, 10 ng/ml CNTF, 10 ng/ml NGF, 10 ng/ml Shh	1. 3 days 2. 7 days 3. 14 days	Retinal ganglion-like cells	microRNA-132
Adipose-derived stem cells <sup>159</sup>	Human	DMEM/F12 medium	1. 2 days 2. 80 days	Retinal pigment epithelial cells	/
Adipose-derived stem cells <sup>168</sup>	Human	1. DMEM, 1% FBS, 2 mM L-glutamine 2. 1 ng/ml Noggin, 1 ng/ml DKK-1, 10 ng/ml IGF-1, 1% N2, 2% Vitamin, 2% B27 3. 10 ng/ml Noggin, 10 ng/ml DKK-1, 10 ng/ml IGF-1, 1% N2, 2% Vitamin B27 4. 5 ng/ml b-FGF, 5 ng/ml IGF-1, 1% N2, 2% Vitamin, 2% B27 5. 100 µM Vitamin A	1. 7 days 2. 7 days 3. 13 days 4. 1 day	Retinal progenitor cells, Photoreceptor cells	/
Adipose-derived stem cells <sup>165</sup>	Human	1. DMEM/F12 medium, 10% knockout serum replacement, 1x B27 supplement, 1 ng/ml noggin, 1 ng/ml Dkk-1, 5 ng/ml IGF-1 2. DMEM/F12 medium, 1x B27 supplement, 1x N2 supplement, 10 ng/ml noggin, 10 ng/ml Dkk-1, 10 ng/ml IGF-1, 5 ng/ml bFGF 3. DMEM/F12 medium, 1x B27 supplement, 1x ITS, 10 ng/ml noggin, 10 ng/ml Dkk-1, 10 ng/ml IGF-1, 5 ng/ml bFGF	1. 3 days 2. 7 days 3. 14 days	Retinal progenitor cells, Retinal ganglion cells, Photoreceptor cells	Notch signaling activation
Spermatogonial stem cells <sup>166</sup>	Mouse	1. Glasgow-MEM, 5% KSR, 0.1 mM NEAA, 0.1 mM pyruvate, and 0.1 mM β-ME 2. DMEM/F-12, N-2 supplement 3. DMEM/F-12, 10% ES-FBS, N-2 supplement, 0.5mM retinoic acid, 30 ng/ml BDNF	1. 7 days 2. 3 days 3. 4 days	Retinal ganglion cells	/

BDNF: brain-derived neurotrophic factor; bFGF: basic fibroblast growth factor; CNTF: ciliary neurotrophic factor; Dkk-1: dickkopf-related protein 1; DMEM: Dulbecco's modified Eagle medium; EGF: epidermal growth factor; FBS: fetal bovine serum; IGF-1: insulin-like growth factor 1; ITS: insulin-transferrin-selenium; ME: mercaptoethanol; MEM: minimal essential medium; MIO-M1: Human Müller cell line; NEAA: non-essential amino acids; NGF: nerve growth factor; Shh: sonic hedgehog.



**Figure 2.** Research progress of adult stem cells in optic neuropathies. Adult stem cells can be conveniently isolated from different tissues and organs, including adipose tissue, bone marrow, and periodontal ligament. Cell replacement therapy (based on the differentiation of adult stem cells into retinal ganglion cells [RGCs]) and the neuroprotective effects of adult stem cells (based on the neurotrophic factor and exosome secretion) have been investigated to alleviate the loss of RGCs and their axons and promote RGC survival and axonal regeneration. RNFL: retinal nerve fiber layer. Parts of the figure were created by Figdraw ([www.figdraw.com](http://www.figdraw.com)).

determined. These questions are critical for future clinical application of adult stem cell therapy, which creates the research opportunities on adult stem cells for RGC protection and regeneration. Furthermore, functional modulation of other cells in the optic nerve, including astrocytes, oligodendrocytes, and microglia, should also be considered in the treatments for optic neuropathies. Further studies are needed to optimize the application of adult stem cells in the treatment regime for optic neuropathy.

### Acknowledgments

We are grateful to Mr. Nikky Yuk-Ki Lai and Miss Samantha Antonio Leong from the Chinese University of Hong Kong for their assistance.

### Author Contributions

S.T. and T.K.N.: Conception and design. S.T. and T.K.N.: Financial support. S.T., Y.Y., Q.Y., X.L.Y., and T.K.N.: Collection and/or assembly of data. Q.Y., X.L.Y., and T.K.N.: Data analysis and interpretation. S.T., Y.Y., Q.Y., X.L.Y., and T.K.N.: Manuscript writing. S.T., Y.Y., L.P.C., and T.K.N.: Critical revision of manuscript.

### Ethical Approval

This study was approved by our institutional review board.

### Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

### Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported in part by the National Natural Science Foundation of China (project code: 81800822 to S.T.), the Joint Regional Basic Science and Applied Basic Science Research Fund of Guangdong Province (project code: 2019A1515110685 to T.K.N.), internal grant from the Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong (project code: 18-001 to T.K.N.), China, and internal research grant from the Hong Kong Polytechnic University (project code: ZVS7 and BD82 to S.T.), Hong Kong.

### ORCID iD

Tsz Kin Ng  <https://orcid.org/0000-0001-7863-7229>

### References

- Behbehani R. Clinical approach to optic neuropathies. *Clin Ophthalmol.* 2007;1(3):233–46.
- Goodwin J. Autoimmune optic neuropathy. *Curr Neurol Neurosci Rep.* 2006;6(5):396–402.
- O'Neill EC, Danesh-Meyer HV, Connell PP, Trounce IA, Coote MA, Mackey DA, Crowston JG. The optic nerve head in acquired optic neuropathies. *Nat Rev Neurol.* 2010;6(4):221–36.
- Shams PN, Plant GT. Optic neuritis: a review. *Int MS J.* 2009;16(3):82–89.
- Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, Nakashima I, Weinshenker BG. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet.* 2004;364(9451):2106–12.
- Kezuka T, Usui Y, Goto H. Analysis of the pathogenesis of experimental autoimmune optic neuritis. *J Biomed Biotechnol.* 2011;2011:294046.
- Zhao Y, Tan S, Chan TCY, Xu Q, Zhao J, Teng D, Fu H, Wei S. Clinical features of demyelinating optic neuritis with seropositive myelin oligodendrocyte glycoprotein antibody in Chinese patients. *Br J Ophthalmol.* 2018;102(10):1372–77.
- Rodriguez M, Siva A, Cross SA, O'Brien PC, Kurland LT. Optic neuritis: a population-based study in Olmsted County, Minnesota. *Neurology.* 1995;45(2):244–50.
- Khan RS, Dine K, Geisler JG, Shindler KS. Mitochondrial uncoupler prodrug of 2,4-dinitrophenol, MP201, prevents neuronal damage and preserves vision in experimental optic neuritis. *Oxid Med Cell Longev.* 2017;2017:7180632.
- Zeng Q, Dong X, Ruan C, Hu B, Luo Y, Luo Z, Xu L, Zhou H, Wang R, Yang H. CD14(+)CD16(++) monocytes are increased in patients with NMO and are selectively suppressed by glucocorticoids therapy. *J Neuroimmunol.* 2016;300:1–8.
- Etemadifar M, Salari M, Mirmosayyeb O, Serati M, Nikkhah R, Askari M, Fayyazi E. Efficacy and safety of rituximab in neuromyelitis optica: review of evidence. *J Res Med Sci.* 2017;22:18.
- Oshiro A, Nakamura S, Tamashiro K, Fujihara K. [Anti-MOG + neuromyelitis optica spectrum disorders treated with plasmapheresis]. *No to Hattatsu.* 2016;48(3):199–203.
- Valentino P, Marnetto F, Granieri L, Capobianco M, Bertolotto A. Aquaporin-4 antibody titration in NMO patients treated with rituximab: a retrospective study. *Neurol Neuroimmunol Neuroinflamm.* 2017;4(2):e317.
- Peng F, Qiu W, Li J, Hu X, Huang R, Lin D, Bao J, Jiang Y, Bian L. A preliminary result of treatment of neuromyelitis optica with autologous peripheral hematopoietic stem cell transplantation. *Neurologist.* 2010;16(6):375–78.
- Lu Z, Ye D, Qian L, Zhu L, Wang C, Guan D, Zhang X, Xu Y. Human umbilical cord mesenchymal stem cell therapy on neuromyelitis optica. *Curr Neurovasc Res.* 2012;9(4):250–55.
- Burt RK, Balabanov R, Han X, Burns C, Gastala J, Jovanovic B, Helenowski I, Jitrapaikulsan J, Fryer JP, Pittock SJ. Autologous nonmyeloablative hematopoietic stem cell transplantation for neuromyelitis optica. *Neurology.* 2019;93(18):e1732–41.
- Weiss JN, Levy S, Malkin A. Stem Cell Ophthalmology Treatment Study (SCOTS) for retinal and optic nerve diseases: a preliminary report. *Neural Regen Res.* 2015;10(6):982–88.
- Fu Y, Yan Y, Qi Y, Yang L, Li T, Zhang N, Yu C, Su L, Zhang R, Shen Y, Lin S, et al. Impact of autologous mesenchymal stem cell infusion on neuromyelitis optica spectrum disorder: a pilot, 2-year observational study. *CNS Neurosci Ther.* 2016;22(8):677–85.
- Weiss JN, Levy S, Benes SC. Stem Cell Ophthalmology Treatment Study (SCOTS) for retinal and optic nerve diseases: a case report of improvement in relapsing auto-immune optic neuropathy. *Neural Regen Res.* 2015;10(9):1507–15.
- Hayreh SS. Retinal and optic nerve head ischemic disorders and atherosclerosis: role of serotonin. *Prog Retin Eye Res.* 1999;18(2):191–221.
- Kerr NM, Chew SS, Danesh-Meyer HV. Non-arteritic anterior ischaemic optic neuropathy: a review and update. *J Clin Neurosci.* 2009;16(8):994–1000.
- Beck RW, Hayreh SS, Podhajsky PA, Tan ES, Moke PS. Aspirin therapy in nonarteritic anterior ischemic optic neuropathy. *Am J Ophthalmol.* 1997;123(2):212–17.
- Kupersmith MJ, Frohman L, Sanderson M, Jacobs J, Hirschfeld J, Ku C, Warren FA. Aspirin reduces the incidence of second eye NAION: a retrospective study. *J Neuroophthalmol.* 1997;17(4):250–53.
- Lee AG, Bioussé V. Should steroids be offered to patients with nonarteritic anterior ischemic optic neuropathy. *J Neuroophthalmol.* 2010;30(2):193–98.
- Goldenberg-Cohen N, Avraham-Lubin BC, Sadikov T, Goldstein RS, Askenasy N. Primitive stem cells derived from bone marrow express glial and neuronal markers and support revascularization in injured retina exposed to ischemic and mechanical damage. *Stem Cells Dev.* 2012;21(9):1488–500.
- Hattenhauer MG, Leavitt JA, Hodge DO, Grill R, Gray DT. Incidence of nonarteritic anterior ischemic optic neuropathy. *Am J Ophthalmol.* 1997;123(1):103–107.
- Puomila A, Hamalainen P, Kivioja S, Savontaus ML, Koivumaki S, Huoponen K, Nikoskelainen E. Epidemiology



- and penetrance of Leber hereditary optic neuropathy in Finland. *Eur J Hum Genet.* 2007;15(10):1079–89.
28. Yu-Wai-Man P, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. The epidemiology of Leber hereditary optic neuropathy in the North East of England. *Am J Hum Genet.* 2003;72(2):333–39.
  29. Weiss JN, Levy S, Benes SC. Stem Cell Ophthalmology Treatment Study (SCOTS): bone marrow-derived stem cells in the treatment of Leber's hereditary optic neuropathy. *Neural Regen Res.* 2016;11(10):1685–94.
  30. Yu-Wai-Man P, Chinnery PF. Dominant optic atrophy: novel OPA1 mutations and revised prevalence estimates. *Ophthalmology.* 2013;120(8):1712–12.
  31. Lenaers G, Hamel C, Delettre C, Amati-Bonneau P, Procaccio V, Bonneau D, Reynier P, Milea D. Dominant optic atrophy. *Orphanet J Rare Dis.* 2012;7:46.
  32. Rigi M, Almarzouqi SJ, Morgan ML, Lee AG. Papilledema: epidemiology, etiology, and clinical management. *Eye Brain.* 2015;7:47–57.
  33. Steinsapir KD, Goldberg RA. Traumatic optic neuropathy. *Surv Ophthalmol.* 1994;38(6): 487–518.
  34. Oh HJ, Yeo DG, Hwang SC. Surgical treatment for traumatic optic neuropathy. *Korean J Neurotrauma.* 2018;14(2):55–60.
  35. Zaverucha-do-Valle C, Gubert F, Bargas-Rega M, Coronel JL, Mesentier-Louro LA, Mencalha A, Abdelhay E, Santiago MF, Mendez-Otero R. Bone marrow mononuclear cells increase retinal ganglion cell survival and axon regeneration in the adult rat. *Cell Transplant.* 2011;20(3):391–406.
  36. Cen LP, Ng TK, Liang JJ, Zhuang X, Yao X, Yam GH, Chen H, Cheung HS, Zhang M, Pang CP. Human periodontal ligament-derived stem cells promote retinal ganglion cell survival and axon regeneration after optic nerve injury. *Stem Cells.* 2018;36(6):844–55.
  37. Mead B, Logan A, Berry M, Leadbeater W, Scheven BA. Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. *Invest Ophthalmol Vis Sci.* 2013;54(12):7544–56.
  38. Tham Y-C, Li X, Wong TY, Quigley HA, Aung T, Cheng C-Y. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology.* 2014;121(11):2081–90.
  39. Jonas JB, Aung T, Bourne RR, Bron AM, Ritch R, Panda-Jonas S. Glaucoma *Lancet.* 2017;390(10108):2183–93.
  40. Yu S, Tanabe T, Dezawa M, Ishikawa H, Yoshimura N. Effects of bone marrow stromal cell injection in an experimental glaucoma model. *Biochem Biophys Res Commun.* 2006;344(4):1071–79.
  41. Johnson TV, Bull ND, Hunt DP, Marina N, Tomarev SI, Martin KR. Neuroprotective effects of intravitreal mesenchymal stem cell transplantation in experimental glaucoma. *Invest Ophthalmol Vis Sci.* 2010;51(4):2051–59.
  42. Mead B, Hill LJ, Blanch RJ, Ward K, Logan A, Berry M, Leadbeater W, Scheven BA. Mesenchymal stromal cell-mediated neuroprotection and functional preservation of retinal ganglion cells in a rodent model of glaucoma. *Cytotherapy.* 2016;18(4):487–96.
  43. Mead B, Amaral J, Tomarev S. Mesenchymal stem cell-derived small extracellular vesicles promote neuroprotection in rodent models of glaucoma. *Invest Ophthalmol Vis Sci.* 2018;59(2):702–14.
  44. Carelli V, Ross-Cisneros FN, Sadun AA. Optic nerve degeneration and mitochondrial dysfunction: genetic and acquired optic neuropathies. *Neurochem Int.* 2002;40(6):573–84.
  45. Yu-Wai-Man P, Votruba M, Moore AT, Chinnery PF. Treatment strategies for inherited optic neuropathies: past, present and future. *Eye.* 2014;28(5):521–37.
  46. O'Neill EC, Mackey DA, Connell PP, Hewitt AW, Danesh-Meyer HV, Crowston JG. The optic nerve head in hereditary optic neuropathies. *Nat Rev Neurol.* 2009;5(5):277–87.
  47. Doucette LP, Rasnitsyn A, Seifi M, Walter MA. The interactions of genes, age, and environment in glaucoma pathogenesis. *Surv Ophthalmol.* 2015;60(4):310–26.
  48. Yao Y, Xu Y, Liang JJ, Zhuang X, Ng TK. Longitudinal and simultaneous profiling of 11 modes of cell death in mouse retina post-optic nerve injury. *Exp Eye Res.* 2022;222:109159.
  49. Liu K, Tedeschi A, Park KK, He Z. Neuronal intrinsic mechanisms of axon regeneration. *Annu Rev Neurosci.* 2011;34: 131–52.
  50. Cen LP, Liang JJ, Chen JH, Harvey AR, Ng TK, Zhang M, Pang CP, Cui Q, Fan YM. AAV-mediated transfer of RhoA shRNA and CNTF promotes retinal ganglion cell survival and axon regeneration. *Neuroscience.* 2017;343:472–82.
  51. Kyhn MV, Klassen H, Johansson UE, Warfvinge K, Lavik E, Kiilgaard JF, Prause JU, Scherfig E, Young M, la Cour M. Delayed administration of glial cell line-derived neurotrophic factor (GDNF) protects retinal ganglion cells in a pig model of acute retinal ischemia. *Exp Eye Res.* 2009; 89(6):1012–20.
  52. Fu L, Fung FK, Lo AC, Chan YK, So KF, Wong IY, Shih KC, Lai JS. Transcorneal electrical stimulation inhibits retinal microglial activation and enhances retinal ganglion cell survival after acute ocular hypertensive injury. *Transl Vis Sci Technol.* 2018;7(3):7.
  53. Thomas CN, Berry M, Logan A, Blanch RJ, Ahmed Z. Caspases in retinal ganglion cell death and axon regeneration. *Cell Death Discov.* 2017;3:17032.
  54. Domeniconi M, Filbin MT. Overcoming inhibitors in myelin to promote axonal regeneration. *J Neurol Sci.* 2005;233(1–2): 43–47.
  55. Osborne A, Khatib TZ, Songra L, Barber AC, Hall K, Kong GY, Widdowson PS, Martin KR. Neuroprotection of retinal ganglion cells by a novel gene therapy construct that achieves sustained enhancement of brain-derived neurotrophic factor/tropomyosin-related kinase receptor-B signaling. *Cell Death Dis.* 2018;9(10):1–18.
  56. Rhee J, Shih KC. Use of gene therapy in retinal ganglion cell neuroprotection: current concepts and future directions. *Biomolecules.* 2021;11(4):581.
  57. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells.* 2006;24(5):1294–301.
  58. Wagers AJ, Weissman IL. Plasticity of adult stem cells. *Cell.* 2004;116(5):639–48.
  59. Lodish H, Flygare J, Chou S. From stem cell to erythroblast: regulation of red cell production at multiple levels by multiple hormones. *IUBMB Life.* 2010;62(7):492–96.
  60. Krause DS, Ito T, Fackler MJ, Smith OM, Collector MI, Sharkis SJ, May WS. Characterization of murine CD34, a marker for hematopoietic progenitor and stem cells. *Blood.* 1994;84(3):691–701.

61. Baumgartner C, Toifl S, Farlik M, Halbritter F, Scheicher R, Fischer I, Sexl V, Bock C, Baccarini M. An ERK-dependent feedback mechanism prevents hematopoietic stem cell exhaustion. *Cell Stem Cell*. 2018;22(6):879–892.
62. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284(5411):143–47.
63. Young HE, Mancini ML, Wright RP, Smith JC, Black AC Jr, Reagan CR, Lucas PA. Mesenchymal stem-cells reside within the connective tissues of many organs. *Dev Dyn*. 1995; 202(2):137–44.
64. Lv FJ, Tuan RS, Cheung KM, Leung VY. Concise review: the surface markers and identity of human mesenchymal stem cells. *Stem Cells*. 2014;32(6):1408–19.
65. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. *Cytotherapy*. 2006;8(4):315–17.
66. Xiang M. Intrinsic control of mammalian retinogenesis. *Cell Mol Life Sci*. 2013;70(14):2519–32.
67. Bi YY, Feng DF, Pan DC. Stem/progenitor cells: a potential source of retina-specific cells for retinal repair. *Neurosci Res*. 2009;65(3):215–21.
68. Tropepe V, Coles BLK, Chiasson BJ, Horsford DJ, Elia AJ, McInnes RR, van der Kooy D. Retinal stem cells in the adult mammalian eye. *Science*. 2000;287(5460):2032–36.
69. Zou T, Gao L, Zeng Y, Li Q, Li Y, Chen S, Hu X, Chen X, Fu C, Xu H, Yin ZQ. Organoid-derived C-Kit(+)/SSEA4(-) human retinal progenitor cells promote a protective retinal microenvironment during transplantation in rodents. *Nat Commun*. 2019;10(1):1205.
70. Cen LP, Ng TK. Stem cell therapy for retinal ganglion cell degeneration. *Neural Regen Res*. 2018;13(8):1352–53.
71. Yi T, Song SU. Immunomodulatory properties of mesenchymal stem cells and their therapeutic applications. *Arch Pharm Res*. 2012;35(2):213–21.
72. Losurdo M, Pedrazzoli M, D'Agostino C, Elia CA, Massenzio F, Lonati E, Mauri M, Rizzi L, Molteni L, Bresciani E, Dander E, et al. Intranasal delivery of mesenchymal stem cell-derived extracellular vesicles exerts immunomodulatory and neuroprotective effects in a 3xTg model of Alzheimer's disease. *Stem Cells Transl Med*. 2020;9(9):1068–84.
73. Fan XL, Zhang Y, Li X, Fu QL. Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy. *Cell Mol Life Sci*. 2020;77(14):2771–94.
74. Jiang D, Gao F, Zhang Y, Wong DS, Li Q, Tse HF, Xu G, Yu Z, Lian Q. Mitochondrial transfer of mesenchymal stem cells effectively protects corneal epithelial cells from mitochondrial damage. *Cell Death Dis*. 2016;7(11):e2467.
75. Feng Y, Zhu R, Shen J, Wu J, Lu W, Zhang J, Zhang J, Liu K. Human bone marrow mesenchymal stem cells rescue endothelial cells experiencing chemotherapy stress by mitochondrial transfer via tunneling nanotubes. *Stem Cells Dev*. 2019;28(10):674–82.
76. Ng TK, Fortino VR, Pelaez D, Cheung HS. Progress of mesenchymal stem cell therapy for neural and retinal diseases. *World J Stem Cells*. 2014;6(2):111–19.
77. Bull ND, Limb GA, Martin KR. Human Muller stem cell (MIO-M1) transplantation in a rat model of glaucoma: survival, differentiation, and integration. *Invest Ophthalmol Vis Sci*. 2008;49(8):3449–56.
78. Kaufman DI, Trobe JD, Eggenberger ER, Whitaker JN. Practice parameter: the role of corticosteroids in the management of acute monosymptomatic optic neuritis—report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*. 2000;54(11):2039–44.
79. Trebst C, Jarius S, Berthele A, Paul F, Schippling S, Wildemann B, Borisow N, Kleiter I, Aktas O, Kümpfel T; Neuromyelitis Optica Study Group (NEMOS). Update on the diagnosis and treatment of neuromyelitis optica: recommendations of the Neuromyelitis Optica Study Group (NEMOS). *J Neurol*. 2014;261(1):1–16.
80. Gramlich OW, Brown AJ, Godwin CR, Chimenti MS, Boland LK, Ankrum JA, Kardon RH. Systemic mesenchymal stem cell treatment mitigates structural and functional retinal ganglion cell degeneration in a mouse model of multiple sclerosis. *Transl Vis Sci Technol*. 2020;9(8):16.
81. Wen YT, Ho YC, Lee YC, Ding DC, Liu PK, Tsai RK. The benefits and hazards of intravitreal mesenchymal stem cell (MSC) based-therapies in the experimental ischemic optic neuropathy. *Int J Mol Sci*. 2021;22(4):2117.
82. Yang C, Yang Y, Ma L, Zhang GX, Shi FD, Yan Y, Chang G. Study of the cytological features of bone marrow mesenchymal stem cells from patients with neuromyelitis optica. *Int J Mol Med*. 2019;43(3):1395–1405.
83. Xu J, Ji BX, Su L, Dong HQ, Sun WL, Wan SG, Liu YO, Zhang P, Liu CY. Clinical outcome of autologous peripheral blood stem cell transplantation in opticospinal and conventional forms of secondary progressive multiple sclerosis in a Chinese population. *Ann Hematol*. 2011;90(3):343–48.
84. Matiello M, Pittock SJ, Porrata L, Weinschenker BG. Failure of autologous hematopoietic stem cell transplantation to prevent relapse of neuromyelitis optica. *Arch Neurol*. 2011; 68(7):953–55.
85. Arnold AC. The 14th Hoyt lecture: ischemic optic neuropathy: the evolving profile, 1966-2015. *J Neuroophthalmol*. 2016;36(2):208–15.
86. Biousse V, Newman NJ. Ischemic optic neuropathies. *N Engl J Med*. 2015;372(25):2428–36.
87. Micieli JA, Lam C, Najem K, Margolin EA. Aqueous humor cytokines in patients with acute nonarteritic anterior ischemic optic neuropathy. *Am J Ophthalmol*. 2017;177:175–81.
88. Katz DM, Trobe JD. Is there treatment for nonarteritic anterior ischemic optic neuropathy. *Curr Opin Ophthalmol*. 2015; 26(6):458–63.
89. Avraham-Lubin BC, Goldenberg-Cohen N, Sadikov T, Askenasy N. VEGF induces neuroglial differentiation in bone marrow-derived stem cells and promotes microglia conversion following mobilization with GM-CSF. *Stem Cell Rev Rep*. 2012;8(4):1199–210.
90. Goldenberg-Cohen N, Avraham-Lubin BCR, Sadikov T, Askenasy N. Effect of coadministration of neuronal growth factors on neuroglial differentiation of bone marrow-derived stem cells in the ischemic retina. *Invest Ophthalmol Vis Sci*. 2014;55(1):502–12.
91. Weiss JN, Levy S, Benes SC. Stem Cell Ophthalmology Treatment Study: bone marrow derived stem cells in the treatment of non-arteritic ischemic optic neuropathy (NAION). *Stem Cell Investig*. 2017;4:94.

92. Negrel AD, Thylefors B. The global impact of eye injuries. *Ophthalmic Epidemiol.* 1998;5(3):143–69.
93. Sharma AK, Aslami AN, Srivastava JP, Iqbal J. Visual outcome of traumatic cataract at a tertiary eye care centre in North India: a prospective study. *J Clin Diagn Res.* 2016;10(1):NC05–18.
94. Cirovic S, Bholra RM, Hose DR, Howard IC, Lawford PV, Marr JE, Parsons MA. Computer modelling study of the mechanism of optic nerve injury in blunt trauma. *Br J Ophthalmol.* 2006;90(6):778–83.
95. Povlishock JT, Christman CW. The pathobiology of traumatically induced axonal injury in animals and humans: a review of current thoughts. *J Neurotrauma.* 1995;12(4):555–64.
96. Mesentier-Louro LA, Zaverucha-do-Valle C, da Silva-Junior AJ, Nascimento-Dos-Santos G, Gubert F, de Figueiredo AB, Torres AL, Paredes BD, Teixeira C, Tovar-Moll F, Mendez-Otero R, et al. Distribution of mesenchymal stem cells and effects on neuronal survival and axon regeneration after optic nerve crush and cell therapy. *PLoS ONE.* 2014;9(10):e110722.
97. da Silva-Junior AJ, Mesentier-Louro LA, Nascimento-Dos-Santos G, Teixeira-Pinheiro LC, Vasques JF, Chimeli-Ormonde L, Bodart-Santos V, de Carvalho LRP, Santiago MF, Mendez-Otero R. Human mesenchymal stem cell therapy promotes retinal ganglion cell survival and target reconnection after optic nerve crush in adult rats. *Stem Cell Res Ther.* 2021;12(1):69.
98. Lyseng-Williamson KA. Idebene: a review in Leber's hereditary optic neuropathy. *Drugs.* 2016;76(7):805–13.
99. Valero T. Mitochondrial biogenesis: pharmacological approaches. *Curr Pharm Des.* 2014;20(35):5507–5509.
100. Lin CS, Sharpley MS, Fan W, Waymire KG, Sadun AA, Carelli V, Ross-Cisneros FN, Baciup P, Sung E, McManus MJ, Pan BX, et al. Mouse mtDNA mutant model of Leber hereditary optic neuropathy. *Proc Natl Acad Sci U S A.* 2012;109(49):20065–70.
101. Yang YP, Nguyen PNN, Lin TC, Yarmishyn AA, Chen WS, Hwang DK, Chiou GY, Lin TW, Chien CS, Tsai CY, Chiou SH, et al. Glutamate stimulation dysregulates AMPA receptors-induced signal transduction pathway in Leber's inherited optic neuropathy patient-specific hiPSC-derived retinal ganglion cells. *Cells.* 2019;8(6):625.
102. Sharma LK, Tiwari M, Rai NK, Bai Y. Mitophagy activation repairs Leber's hereditary optic neuropathy-associated mitochondrial dysfunction and improves cell survival. *Hum Mol Genet.* 2019;28(3):422–33.
103. McMonnies CW. Glaucoma history and risk factors. *J Optom.* 2017;10(2):71–78.
104. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA.* 2014;311(18):1901–11.
105. Orgül S. Compartment syndrome in the optic nerve: a new hypothesis in the pathogenesis of glaucoma. *Acta Ophthalmol.* 2012;90(7):686–89.
106. Li S, Zhang A, Cao W, Sun X. Elevated plasma endothelin-1 levels in normal tension glaucoma and primary open-angle glaucoma: a meta-analysis. *J Ophthalmol.* 2016;2016:2678017.
107. Shoeb Ahmad S, Abdul Ghani S, Hemalata Rajagopal T. Current concepts in the biochemical mechanisms of glaucomatous neurodegeneration. *J Curr Glaucoma Pract.* 2013;7(2):49–53.
108. Yang Y, Xu C, Chen Y, Liang JJ, Xu Y, Chen SL, Huang S, Yang Q, Cen LP, Pang CP, Sun XH, et al. Green tea extract ameliorates ischemia-induced retinal ganglion cell degeneration in rats. *Oxid Med Cell Longev.* 2019;2019:8407206.
109. Su W, Li Z, Jia Y, Zhu Y, Cai W, Wan P, Zhang Y, Zheng SG, Zhuo Y. microRNA-21a-5p/PDCD4 axis regulates mesenchymal stem cell-induced neuroprotection in acute glaucoma. *J Mol Cell Biol.* 2017;9(4):289–301.
110. Mead B, Tomarev S. Bone marrow-derived mesenchymal stem cells-derived exosomes promote survival of retinal ganglion cells through miRNA-dependent mechanisms. *Stem Cells Transl Med.* 2017;6(4):1273–85.
111. Vilela CAP, Messias A, Calado RT, Siqueira RC, Silva MJL, Covas DT, Paula JS. Retinal function after intravitreal injection of autologous bone marrow-derived mesenchymal stromal cells in advanced glaucoma. *Doc Ophthalmol.* 2021;143(1):33–38.
112. Ng TK, Chen CB, Xu C, Xu Y, Yao X, Huang L, Liang JJ, Cheung HS, Pang CP, Huang Y. Attenuated regenerative properties in human periodontal ligament-derived stem cells of older donor ages with shorter telomere length and lower SSEA4 expression. *Cell Tissue Res.* 2020;381:71–81.
113. Ng TK, Carballosa CM, Pelaez D, Wong HK, Choy KW, Pang CP, Cheung HS. Nicotine alters MicroRNA expression and hinders human adult stem cell regenerative potential. *Stem Cells Dev.* 2013;22(5):781–90.
114. Xu Y, Malladi P, Chiou M, Bekerman E, Giaccia AJ, Longaker MT. In vitro expansion of adipose-derived adult stromal cells in hypoxia enhances early chondrogenesis. *Tissue Eng.* 2007;13(12):2981–93.
115. Martin-Rendon E, Hale SJ, Ryan D, Baban D, Forde SP, Roubelakis M, Sweeney D, Moukayed M, Harris AL, Davies K, Watt SM. Transcriptional profiling of human cord blood CD133+ and cultured bone marrow mesenchymal stem cells in response to hypoxia. *Stem Cells.* 2007;25(4):1003–12.
116. Kanichai M, Ferguson D, Prendergast PJ, Campbell VA. Hypoxia promotes chondrogenesis in rat mesenchymal stem cells: a role for AKT and hypoxia-inducible factor (HIF)-1 $\alpha$ . *J Cell Physiol.* 2008;216(3):708–15.
117. Welford SM, Bedogni B, Gradin K, Poellinger L, Broome Powell M, Giaccia AJ. HIF1 $\alpha$  delays premature senescence through the activation of MIF. *Genes Dev.* 2006;20(24):3366–71.
118. Park SM, Li Q, Ryu MO, Nam A, An JH, Yang JI, Kim SM, Song WJ, Youn HY. Preconditioning of canine adipose tissue-derived mesenchymal stem cells with deferoxamine potentiates anti-inflammatory effects by directing/reprogramming M2 macrophage polarization. *Vet Immunol Immunopathol.* 2020;219:109973.
119. Hu Y, Chen W, Wu L, Jiang L, Qin H, Tang N. Hypoxic preconditioning improves the survival and neural effects of transplanted mesenchymal stem cells via CXCL12/CXCR4 signalling in a rat model of cerebral infarction. *Cell Biochem Funct.* 2019;37(7):504–15.
120. Bianchi G, Banfi A, Mastrogiacomo M, Notaro R, Luzzatto L, Cancedda R, Quarto R. Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2. *Exp Cell Res.* 2003;287(1):98–105.



121. Chiou M, Xu Y, Longaker MT. Mitogenic and chondrogenic effects of fibroblast growth factor-2 in adipose-derived mesenchymal cells. *Biochem Biophys Res Commun.* 2006; 343(2):644–52.
122. Yu Y, Yoo SM, Park HH, Baek SY, Kim YJ, Lee S, Kim YL, Seo KW, Kang KS. Preconditioning with interleukin-1 beta and interferon-gamma enhances the efficacy of human umbilical cord blood-derived mesenchymal stem cells-based therapy via enhancing prostaglandin E2 secretion and indoleamine 2,3-dioxygenase activity in dextran sulfate sodium-induced colitis. *J Tissue Eng Regen Med.* 2019;13:1792–804.
123. Saberi K, Pasbakhsh P, Omid A, Borhani-Haghighi M, Nekoonam S, Omid N, Ghasemi S, Kashani IR. Melatonin preconditioning of bone marrow-derived mesenchymal stem cells promotes their engraftment and improves renal regeneration in a rat model of chronic kidney disease. *J Mol Histol.* 2019;50(2):129–40.
124. Khatlani T, Algudiri D, Alenzi R, Al Subayyil AM, Abomaray FM, Bahattab E, AlAskar AS, Kalionis B, El-Muzaini MF, Abumaree MH. Preconditioning by hydrogen peroxide enhances multiple properties of human decidua basalis mesenchymal stem/multipotent stromal cells. *Stem Cells Int.* 2018;2018:6480793.
125. Zhang F, Peng W, Zhang J, Dong W, Yuan D, Zheng Y, Wang Z. New strategy of bone marrow mesenchymal stem cells against oxidative stress injury via Nrf2 pathway: oxidative stress preconditioning. *J Cell Biochem.* 2019;120(12):19902–14.
126. Cai CX, Teng L, Vu D, He JQ, Guo YR, Li QH, Tang XL, Rokosh G, Bhatnagar A, Bolli R. The heme oxygenase 1 inducer (CoPP) protects human cardiac stem cells against apoptosis through activation of the extracellular signal-regulated Kinase (ERK)/NRF2 signaling pathway and cytokine release. *J Biol Chem.* 2012;287(40):33720–32.
127. Sung DK, Sung SI, Ahn SY, Chang YS, Park WS. Thrombin preconditioning boosts biogenesis of extracellular vesicles from mesenchymal stem cells and enriches their cargo contents via protease-activated receptor-mediated signaling pathways. *Int J Mol Sci.* 2019;20(12):2899.
128. Liu C, Li Y, Yang Z, Zhou Z, Lou Z, Zhang Q. Kartogenin enhances the therapeutic effect of bone marrow mesenchymal stem cells derived exosomes in cartilage repair. *Nanomedicine.* 2020;15(3):273–88.
129. Tsai LK, Leng Y, Wang Z, Leeds P, Chuang DM. The mood stabilizers valproic acid and lithium enhance mesenchymal stem cell migration via distinct mechanisms. *Neuropsychopharmacology.* 2010;35(11):2225–37.
130. Yang Q, Leong SA, Chan KP, Yuan XL, Ng TK. Complex effect of continuous curcumin exposure on human bone marrow-derived mesenchymal stem cell regenerative properties through matrix metalloproteinase regulation. *Basic Clin Pharmacol Toxicol.* 2021;128(1):141–53.
131. Bo H, He J, Wang X, Du R, Bei H, Chen J, Wu F, Zhang W, Chen Q. Danggui Buxue Tang promotes the adhesion and migration of bone marrow stromal cells via the focal adhesion pathway in vitro. *J Ethnopharmacol.* 2019;231:90–97.
132. Wang JY, Chen WM, Wen CS, Hung SC, Chen PW, Chiu JH. Du-Huo-Ji-Sheng-Tang and its active component Ligusticum chuanxiong promote osteogenic differentiation and decrease the aging process of human mesenchymal stem cells. *J Ethnopharmacol.* 2017;198:64–72.
133. Kim R, Lee S, Lee CY, Yun H, Lee H, Lee MY, Kim J, Jeong JY, Baek K, Chang W. *Salvia miltiorrhiza* enhances the survival of mesenchymal stem cells under ischemic conditions. *J Pharm Pharmacol.* 2018;70(9):1228–41.
134. Wang X, Liu C, Xu Y, Chen P, Shen Y, Xu Y, Zhao Y, Chen W, Zhang X, Ouyang Y, Wang Y, et al. Combination of mesenchymal stem cell injection with icariin for the treatment of diabetes-associated erectile dysfunction. *PLoS ONE.* 2017;12(3):e0174145.
135. Zhao ZY, Yang L, Mu X, Xu L, Yu X, Jiao Y, Zhang X, Fu L. *Cajanine* promotes osteogenic differentiation and proliferation of human bone marrow mesenchymal stem cells. *Adv Clin Exp Med.* 2019;28(1):45–50.
136. Chen XJ, Shen YS, He MC, Yang F, Yang P, Pang FX, He W, Cao YM, Wei QS. Polydatin promotes the osteogenic differentiation of human bone mesenchymal stem cells by activating the BMP2-Wnt/beta-catenin signaling pathway. *Biomed Pharmacother.* 2019;112:108746.
137. Soares DM, Ginani F, Henriques AG, Barboza CAG. Effects of laser therapy on the proliferation of human periodontal ligament stem cells. *Lasers Med Sci.* 2015;30(3):1171–74.
138. Wang YH, Wu JY, Kong SC, Chiang MH, Ho ML, Yeh ML, Chen CH. Low power laser irradiation and human adipose-derived stem cell treatments promote bone regeneration in critical-sized calvarial defects in rats. *PLoS ONE.* 2018;13(4):e0195337.
139. Liao X, Li SH, Xie GH, Xie S, Xiao LL, Song JX, Liu HW. Preconditioning with low-level laser irradiation enhances the therapeutic potential of human adipose-derived stem cells in a mouse model of photoaged skin. *Photochem Photobiol.* 2018;94(4):780–90.
140. Ning T, Guo J, Zhang K, Li K, Zhang J, Yang Z, Ge Z. Nanosecond pulsed electric fields enhanced chondrogenic potential of mesenchymal stem cells via JNK/CREB-STAT3 signaling pathway. *Stem Cell Res Ther.* 2019;10(1):45.
141. George PM, Bliss TM, Hua T, Lee A, Oh B, Levinson A, Mehta S, Sun G, Steinberg GK. Electrical preconditioning of stem cells with a conductive polymer scaffold enhances stroke recovery. *Biomaterials.* 2017;142:31–40.
142. Jahanshahi A, Schonfeld L, Janssen ML, Heschem S, Kocabicak E, Steinbusch HW, van Overbeeke JJ, Temel Y. Electrical stimulation of the motor cortex enhances progenitor cell migration in the adult rat brain. *Exp Brain Res.* 2013;231(2):165–77.
143. Morimoto J, Yasuhara T, Kameda M, Umakoshi M, Kin I, Kuwahara K, Kin K, Okazaki M, Takeuchi H, Sasaki T, Toyoshima A, et al. Electrical stimulation enhances migratory ability of transplanted bone marrow stromal cells in a rodent ischemic stroke model. *Cell Physiol Biochem.* 2018; 46(1):57–68.
144. Hiram Y, Osakada F, Takahashi K, Okita K, Yamanaka S, Ikeda H, Yoshimura N, Takahashi M. Generation of retinal cells from mouse and human induced pluripotent stem cells. *Neurosci Lett.* 2009;458(3):126–31.
145. Kobayashi W, Onishi A, Tu HY, Takihara Y, Matsumura M, Tsujimoto K, Inatani M, Nakazawa T, Takahashi M. Culture systems of dissociated mouse and human pluripotent stem cell-derived retinal ganglion cells purified



- by two-step immunopanning. *Invest Ophthalmol Vis Sci.* 2018;59(2):776–87.
146. Wu YR, Wang AG, Chen YT, Yarmishyn AA, Buddhakosai W, Yang TC, Hwang DK, Yang YP, Shen CN, Lee HC, Chiou SH, et al. Bioactivity and gene expression profiles of hiPSC-generated retinal ganglion cells in MT-ND4 mutated Leber's hereditary optic neuropathy. *Exp Cell Res.* 2018;363(2):299–309.
147. Chen J, Riazifar H, Guan MX, Huang T. Modeling autosomal dominant optic atrophy using induced pluripotent stem cells and identifying potential therapeutic targets. *Stem Cell Res Ther.* 2016;7:2.
148. Too LK, Simunovic MP. Retinal stem/progenitor cells derived from adult müller glia for the treatment of retinal degeneration. *Front Cell Dev Biol.* 2021;9:749131.
149. Choi SW, Shin JH, Kim JJ, Shin TH, Seo Y, Kim HS, Kang KS. Direct cell fate conversion of human somatic stem cells into cone and rod photoreceptor-like cells by inhibition of microRNA-203. *Oncotarget.* 2016;7(27):42139–49.
150. Angbohang A, Wu N, Charalambous T, Eastlake K, Lei Y, Kim YS, Sun XH, Limb GA. Downregulation of the canonical WNT signaling pathway by TGFbeta1 inhibits photoreceptor differentiation of adult human müller glia with stem cell characteristics. *Stem Cells Dev.* 2016;25(1):1–12.
151. Choi SW, Kim JJ, Seo MS, Park SB, Kang TW, Lee JY, Lee BC, Kang I, Shin TH, Kim HS, Yu KR, et al. miR-410 Inhibition Induces RPE differentiation of amniotic epithelial stem cells via overexpression of OTX2 and RPE65. *Stem Cell Rev Rep.* 2015;11(3):376–86.
152. Yue X, Zhifeng G, Biyu S, Guofeng X, Tianqiu Z, Jinxia J, Jing X, Suzhe L, Man L, Wei T, Guijuan F, et al. Roles of Wnt/beta-catenin signaling in retinal neuron-like differentiation of bone marrow mesenchymal stem cells from nonobese diabetic mice. *J Mol Neurosci.* 2013;49(2):250–61.
153. Pesaresi M, Bonilla-Pons SA, Simonte G, Sanges D, Di Vicino U, Cosma MP. Endogenous mobilization of bone-marrow cells into the murine retina induces fusion-mediated reprogramming of müller glia cells. *EBioMedicine.* 2018;30:38–51.
154. Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Frisen J. Neurogenesis in the striatum of the adult human brain. *Cell.* 2014;156(5):1072–83.
155. Kurimoto Y, Shibuki H, Kaneko Y, Ichikawa M, Kurokawa T, Takahashi M, Yoshimura N. Transplantation of adult rat hippocampus-derived neural stem cells into retina injured by transient ischemia. *Neurosci Lett.* 2001;306(1–2):57–60.
156. Guo Y, Saloupis P, Shaw SJ, Rickman DW. Engraftment of adult neural progenitor cells transplanted to rat retina injured by transient ischemia. *Invest Ophthalmol Vis Sci.* 2003;44(7):3194–3201.
157. Liu G, David BT, Trawczynski M, Fessler RG. Advances in pluripotent stem cells: history, mechanisms, technologies, and applications. *Stem Cell Rev Rep.* 2020;16(1):3–22.
158. Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer.* 2011;11(4):268–77.
159. Aboutaleb Kadkhodaeian H, Salati A, Lashay A. High efficient differentiation of human adipose-derived stem cells into retinal pigment epithelium-like cells in medium containing small molecules inducers with a simple method. *Tissue Cell.* 2019;56:52–59.
160. Mathivanan I, Trepp C, Brunold C, Baerlocher G, Enzmann V. Retinal differentiation of human bone marrow-derived stem cells by co-culture with retinal pigment epithelium in vitro. *Exp Cell Res.* 2015;333(1):11–20.
161. Xu C, Lu H, Li F, Su G. Protein expression profile on differentiation of bone marrow mesenchymal stem cells into retinal ganglion-like cells. *J Comput Biol.* 2020;27(8):1329–36.
162. Huang L, Liang JJ, Geng YQ, Tsang WM, Yao XW, Jhanji V, Zhang MZ, Cheung HS, Pang CP, Yam GHF. Directing adult human periodontal ligament-derived stem cells to retinal fate. *Invest Ophthalmol Vis Sci.* 2013;54(6):3965–74.
163. Ng TK, Huang L, Cao D, Yip YW, Tsang WM, Yam GH, Pang CP, Cheung HS. Cigarette smoking hinders human periodontal ligament-derived stem cell proliferation, migration and differentiation potentials. *Sci Rep.* 2015;5:7828.
164. Ng TK, Yang Q, Fortino VR, Lai NY, Carballosa CM, Greenberg JM, Choy KW, Pelaez D, Pang CP, Cheung HS. MicroRNA-132 directs human periodontal ligament-derived neural crest stem cell neural differentiation. *J Tissue Eng Regen Med.* 2019;13(1):12–24.
165. Huang Y, Ng TK, Chen CB, Huang B, Liang J, Pang CP, Zhang M. Notch signaling activation enhances human adipose-derived stem cell retinal differentiation. *Stem Cells Int.* 2018;2018:9201374.
166. Suen HC, Qian Y, Liao J, Luk CS, Lee WT, Ng JKW, Chan TTH, Hou HW, Li I, Li K, Chan WY, et al. Transplantation of retinal ganglion cells derived from male germline stem cell as a potential treatment to glaucoma. *Stem Cells Dev.* 2019;28:1365–75.
167. Pelaez D, Huang CY, Cheung HS. Isolation of pluripotent neural crest-derived stem cells from adult human tissues by connexin-43 enrichment. *Stem Cells Dev.* 2013;22(21):2906–14.
168. Xu WW, Huang L, Chong KK, Leung DS, Li BF, Yin ZQ, Huang YF, Pang CP. Differentiation potential of human adipose tissue derived stem cells into photoreceptors through explants culture and enzyme methods. *Int J Ophthalmol.* 2017;10(1):23–29.
169. Kuriyan AE, Albin TA, Townsend JH, Rodriguez M, Pandya HK, Leonard RE 2nd, Parrott MB, Rosenfeld PJ, Flynn HW Jr, Goldberg JL. Vision loss after intravitreal injection of autologous “stem cells” for AMD. *N Engl J Med.* 2017;376(11):1047–53.
170. Tassoni A, Gutteridge A, Barber AC, Osborne A, Martin KR. Molecular mechanisms mediating retinal reactive gliosis following bone marrow mesenchymal stem cell transplantation. *Stem Cells.* 2015;33(10):3006–16.
171. Zhang KY, Tuffy C, Mertz JL, Quillen S, Wechsler L, Quigley HA, Zack DJ, Johnson TV. Role of the internal limiting membrane in structural engraftment and topographic spacing of transplanted human stem cell-derived retinal ganglion cells. *Stem Cell Reports.* 2021;16(1):149–67.
172. Zhou H, Su J, Hu X, Zhou C, Li H, Chen Z, Xiao Q, Wang B, Wu W, Sun Y, Zhou Y, et al. Glia-to-neuron conversion by CRISPR-CasRx alleviates symptoms of neurological disease in mice. *Cell.* 2020;181(3):590–603.