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INVITED REVIEW

Neuro-rejuvenation for neuronal function

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Abstract

Neurodegenerative eye diseases, such as glaucoma, cause irreversible vision loss in millions of patients worldwide, creating serious medical, economic and social issues. Like other mammalian central nervous system tracts, optic nerve intrinsically lacks the capacity for axonal growth and its surrounding environment is also non-permissive to regeneration. Any axonal damage also triggers a vicious cycle of retinal ganglion cell (RGC) death. Exploring methods that can enhance RGCs survival and promote axonal regeneration will not only enable vision restoration for millions of patients, but also shed light on the treatment of other neurodegenerative diseases. In this review article, we will go through three current approaches to cure neurodegenerative eye diseases, including cell based therapy, neuro-regeneration and neuro-rejuvenation.

Key Words: *neurogenerative eye disease; stem cell therapy; neuro-regeneration; neuro-rejuvenation; recovery of vision function*

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Introduction

Vision is one of the most precious senses for humans. Thus, vision loss is a major medical, economic, safety, and social issue affecting millions of persons worldwide. Neurodegenerative diseases of the retina involve progressive and irreversible loss of retinal neurons, their connections and supporting glia, thereby leading to impaired visual function. Major neurodegenerative eye diseases include glaucoma and age-related macular degeneration. Despite being major causes of disability, limited effective therapeutic methods for treating these neurodegenerative eye diseases exist which, at best, slow down the neurodegenerative progression. Until now, no therapeutic option has been proven to successfully reverse or cure vision loss from these neurodegenerative eye diseases, especially glaucoma.

Although glaucoma is a group of aetiologically heterogeneous optic neuropathies, all glaucomas are associated with dysfunction and the eventual death of retinal ganglion cells (RGCs), resulting in degeneration of optic nerve and ultimately loss of vision. Therefore, preserving the RGCs and the optic nerve, both structurally and functionally, is vital for the treatment and possible cure of glaucoma and other neurodegenerative eye diseases. However, similar to other central nervous system (CNS) tracts in mammals, the injured optic nerve has very limited regenerative capacity due to the weak intrinsic capacity of adult neurons to reactivate a growth program after injury and the presence of an inhibitory neuronal tissue environment in the adult CNS.

In order to slow the progressive decline in visual function caused by neurodegenerative eye diseases or even restore vision function, a lot of effort has been devoted toward three major avenues of engagement: cell based therapy, neuro-regeneration and neuro-rejuvenation, which will be discussed in detail later. A Better understanding of these approaches will provide insight into the cure of neurodegenerative eye diseases and bring hope to those patients who suffer from vision loss.

Approaches

Three general approaches have been commonly suggested to overcome the barrier of neuronal repair in the ocular system (**Figure 1**). One approach involves introducing stem cells into the degenerating retina to replace dead neuronal cells. Alternatively, the retinal neuronal growth program might be stimulated in adult neurons, allowing them to overcome the inhibitory environment and grow new dendrites and axon – often termed neuro-regeneration. And last, we propose what we believe to be the most promising and practical approach: enhancing the function of existing RGCs with established visual neuropathways– which we term neuro-rejuvenation.

Most current ocular cell transplantation treatments function as paracrine-mediated therapies in which retinal cell-derived trophic factors protect injured endogenous retinal neurons (*i.e.,* retinal pigmented epithelium cell transplantation; **Figure 1**). However, a successful stem cell

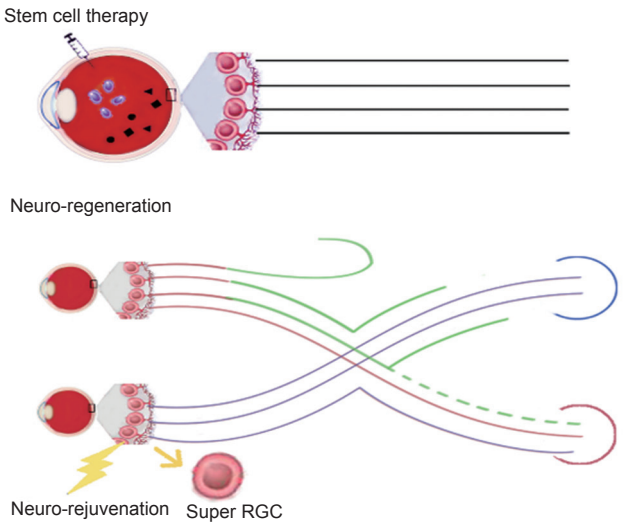

Figure 1 Pathways for neuronal repair.
Stem cell therapies function as paracrine-mediated treatments with trophic factors (black symbols) secreted from transplanted cells (blue cells) protecting injured retinal ganglion cells (RGC, red cells). However, most of injected cells remain in the vitreous cavity and do not or poorly integrate into existing neuronal circuits. Neuro-regeneration involves stimulating intrinsic growth programs in adult neurons to overcome inhibitory environmental cues in the CNS. High levels of local growth stimulation can result in unexpected regeneration patterns. The injured optic nerve can be stimulated to produce new axonal fibers which migrate incorrectly toward the retina and toward wrong targets. The green newly formed axon ends in an ipsilateral tract which is supposed to join the contralateral tract (solid green dots). Neuro-rejuvenation enhances the function of existing RGCs with established neuropathways by stimulating the creation of "super RGCs". Increased neuronal activity and communication among the existing RGCs can enhance signal to noise ratios and retinal ganglion cell function and neuronal survival. CNS: Central nervous system.

treatment for neurodegenerative eye diseases must include not only the replacement of degenerated neurons, but also the correct restoration of neural circuits in a one-to-one correspondence. One major hindrance to successful retinal stem cell transplantation is that differentiation of stem cells into retinal neurons from transplanted stem cells has not been widely achieved *in vivo*, much less restoring a correct neural circuity. For example, RGC cell death protection and neurite growth, induced by bone mesenchymal stem cells (BMSCs), were reported to be mediated by platelet-derived growth factor (PDGF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) (Mead et al., 2013; Johnson et al., 2014). Transplantation of BMSCs into the vitreous cavity after optic nerve transection demonstrated no evidence of their differentiation into mature retinal neurons and BMSCs remained static in the vitreous instead of integrating into the RGC layer. Transplanted cells that do not integrate into the retina cannot communicate with existing neural circuits and have only relatively short lifespans without cell to cell contact or cellular adherence. Thus, the beneficial effect of transplanted stem cells is unlikely to be long lasting, if any.

Embryonic stem cells (ESCs), which can successfully express mature RGC markers after intravitreal injection into N-Methyl-D-aspartic acid (NMDA) damaged retinas (Aoki et al., 2008), are also potential candidates for stem cell therapy. However, only a small percentage of transplanted ESCs appear to integrate into the RGC layer. Most ESCs appear to form abnormal structures, including teratomas and multi-RGC layers, and no visual evoked potentials (VEPs) were detected in NMDA-exposed animals after ESCs transplantation treatment suggesting transplanted ESCs are few or unable to produce any electrical signals upon intravitreal injection. In addition, transplanting ESCs are not equivalent to transplanting differentiated adult retinal cells for cellular transplantation and replacement.

Aside from the physical barriers of transplanting retinal cells, a major and critical challenge that needs to be addressed with stem cell therapy is the altered retinal system caused by the degenerative neuronal disease (Cuenca et al., 2014). Changes to the resting state of the damaged retina may exist, which would affect the function of transplanted cells and the existing cells. For example, the ocular system has immune privilege which can be compromised in disease states. An important aspect of cellular transplantation into the eye that needs to be taken into serious consideration is the possibility of immune rejection and the development of sight threatening uveitis. Transplanted stem cells may also differentiate into unpredictable phenotypes under disease states, unlike phenotypes characterized *in vitro*, and may express abnormal cytokine profiles when surrounded by different retinal cell types unlike the uniform cell types observed *in vitro* under laboratory and non-physiological conditions. Also, uncontrolled cell growth and migration within the CNS neuropil and possible oncogenesis (due to the loss of normal growth and regulatory pathways in stem cells) possible challenges for stem cell therapy the protection and/or restoration of vision loss in neurodegenerative eye diseases.

The injured optic nerve, in glaucoma patients for example, cannot intrinsically regenerate (Quigley, 1999). The lack of adequate stimulation and growth responses by adult neurons to overcome inhibitory molecules in the CNS tissue are the important impediments to axonal regrowth. Overstimulating the retinal growth program may overcome this barrier and alleviate the decline toward retinal cell death signals. Two key pathways have been identified to be able to promote significant axonal growth *in vivo*.

Upregulation of ciliary neurotrophic factor (CNTF) or activation of downstream signal transducer and activator of transcription 3 (STAT3) in adult RGCs after injury was sufficient to promote robust axonal regeneration *in vivo* (Muller et al., 2007; Pernet et al., 2013b). Both molecular approaches elicited long-distance growth of injured optic nerves, near or up to the optic chiasm. The effect of upregulated CNTF can

be further enhanced by the elevation of cyclic AMP (cAMP) (Cui et al., 2003). deletion of the suppressor of cytokine signaling (SOCS)3, which is involved in the negative feedback loop of Stat3 activation, was also able to achieve comparable extent of axonal growth (Smith et al., 2009). Mammalian target of rapamycin (mTOR) is a central regulator of growth in all cell types. Deletion of two upstream repressors - phosphatase and tensin homolog (PTEN) and tuberous sclerosis (TSC)1/2 - selectively in retinal ganglion cells after optic nerve crush strongly stimulated axonal regeneration up to 4 mm (Park et al., 2008; Liu et al., 2010). Synergistic treatment that inducing controlled inflammatory response in the eye, when combined with elevation of intracellular cAMP and deletion of the gene encoding PTEN enabled RGCs to regenerate axons to the full length of the optic nerve in mature mice; even a rare subset of axons managed to enter the brain (Kurimoto et al., 2010). However, under such high level of local growth stimulation, massive axonal sprouts formed and grew randomly in a dense plexus that covered the inner surface of the retina and blood vessels (Pernet et al., 2013a). Unfortunately, an unexpected regeneration pattern was also observed in the injured optic nerve: the return of many new fibers toward the retina and pronounced axonal misrouting at the optic chiasm (**Figure 1**). In the Stat3 group, 40% of axons showed U-turns, and this was also observed in co-activation of mTOR and Stat3 (Pernet et al., 2013a; Luo et al., 2013). More than half of all fibers joined the ipsilateral tract in the PTEN and SOCS3 double-knock out mice, whereas the percentage should be lower than 5% in normal mice (Luo et al., 2013). Vision function recovery requires re-innervation of the correct corresponding region in the occipital cortex by the appropriate types of retinal axons. However, this is not achieved by existing stimulation methods. Even if some of the few regenerated fibers extended beyond the chiasm and terminated in the vicinity of the closest target, it is still doubtful that they will reach the correct target (Luo et al., 2013) (**Figure 1**). Vision would be much worse if connections are made to a wrong target and present a false image – similar to cell phones with the wrong IP addresses communicating with the wrong cell towers. One may have a high intensity signal but at the cost of fidelity.

Compared to the other two approaches for restoring vision, neuro-rejuvenation is the most practical and most accessible approach to preserve and enhance vision by taking advantage of existing neuronal connections to produce an enhanced true image, protect retinal ganglion cells through elevated intrinsic activity which enhances signal to noise ratios, and enhance retinal ganglion cell function (**Figure 1**). Instead of focusing on rescuing dying retinal neurons or replacing them with transplanted neurons, the existing dysfunctional and healthy neurons and neuronal networks are the main therapeutic target. One major technical barrier to neuronal transplantation is the lack of the correct neuronal circuitry through each of the relays (*i.e.*, superior colliculus, lateral geniculate nucleus, occipital cortex), to produce a real image *versus* many bright spots that do not correspond to an image, but only to the location of the transplanted cells – assuming they can even make connections as far as the optic chiasm.

Neuro-rejuvenation is based upon the concept that electrical activity is essential for neuronal survival and axonal growth. Trans-corneal electrical stimulation (TES) promotes both axonal regeneration and survival of RGCs after optic nerve crush (Tagami et al., 2009). Axon regeneration mediated by insulin-like growth factor (IGF-1) receptors can be blocked by IGF-1 receptor antagonists. Furthermore, visual evoked potential (VEP) amplitudes impaired by optic nerve crush can be increased by TES stimulation, indicating functional visual recovery (Miyake et al., 2007). However, compared to traditional electrical stimulation which causes unintended side effects or even may be detrimental to neighboring cells, optogenetic stimulation has significant advantages, such as cell-specific targeting and excellent spatiotemporal resolution reaching millisecond-timescales of activation. Optogenetics combines the use of genetic and optical techniques to modulate activity in targeted cells or tissues. By genetically introducing wavelength-specific modular light-gated ion channels and pumps to targeted neurons, optogenetics offers a noninvasive method of excitation and suppression in existing correctly neuronally linked excitable cells and tissues *in vivo*. Recently, different groups have achieved neuronal protection and axonal regeneration by optogenetic stimulation under various conditions, which offers a promising and practical approach to protect vision loss in neurodegenerative eye diseases, not through cell replacement, but by enhancing the function of existing neuronal cells.

Subcellular calcium release by ryanodine receptor (RyR) is required for neuronal regeneration in *C. elegans* and this can be enhanced by optogenetic stimulation (Sun et al., 2014). Damaged neurons were depolarized by the activation of channelrhodopsin-2 (ChR2), resulting in calcium influx *via* L-type voltage gated calcium channels (VGCCs). The increased calcium flux triggered additional calcium concentration elevation from the endoplasmic reticulum *via* RyR channels, effectively amplifying the innate calcium signal to enhance axonal regeneration. Optogenetic stimulation has also been shown to successfully promote nerve growth in dorsal root ganglion (DRG) cell cultures, which is attributed to the increased secretion of NGF and BDNF (Park et al., 2015). This was verified by the directional bias in the outgrowth of wild-type DRGs in the presence of stimulated ChR2-DRGs. Recently, optically-induced neuronal activity has been demonstrated to sufficiently promote functional motor axon regeneration *in vivo*. Thy1-ChR2-YFP transgenic mice, in which a subset of motor neurons expresses ChR2, received blue light stimulation immediately prior to transection and surgical repair

of the sciatic nerve. Compared to animals without blue light stimulation, mice that received optogenetic stimulation had significantly more ChR2+ axons successfully re-innervated, more reformation of neuromuscular junctions, more moto-neurons that can be retrograde labeled by cholera toxin subunit B, and restored evoked muscle electromyography (EMG) activity (M responses) one month after injury to the gastrocnemius muscle (Ward et al., 2016).

Conclusion

Novel therapeutic approaches to neurodegenerative eye diseases, which cause serious medical, economic and social issues, are very much needed. Thus, far, the three treatment strategies are stem cell transplantation, neuro-regeneration and neuro-rejuvenation, which have all attracted much attention. Compared to stem cell therapy, in which transplanted cells have uncertain cell fates and a challenging integration into neuronal circuits, and neuro-regeneration, which faces challenges of reaching the correct neuronal destinations, neuro-rejuvenation provides us with a more promising and practical approach by taking advantage of existing RGCs and neuronal networks. Instead of introducing new cells into the retina or stimulating those dying retinal neurons, neuro-rejuvenation focuses on existing cells, by enhancing their intrinsic functional capacity, allowing existing neuronal cells to aid their sick partners, and strengthening existing connections to increase existing signal-to-noise ratios. A deeper understanding of neuro-rejuvenation will shed light on the treatment of neurodegenerative eye diseases and bring hope to those patients who suffer from vision loss.

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