MicroRNA-based therapeutics for optic neuropathy: opportunities and challenges

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Optic nerve degeneration is a major cause of irreversible blindness worldwide with glaucoma being the most common optic neuropathy, affecting approximately 76 million people worldwide in 2020. The optic nerve comprises axons of retinal ganglion cells (RGCs), the output neurons of the inner retina. Protecting RGCs and axons from degeneration and regenerating RGC axons to preserve and recover vision in patients with progressive optic neuropathy is an unmet need. Unlike embryonic neurons, mature neurons of the mammalian central nervous system fail to regenerate their axons following injury. The age-related loss of axon regenerative capacity of RGCs over time renders vision loss from optic neuropathy irreversible. The failure of injured RGCs to regenerate axons is largely attributed to inhibitory molecules in the extrinsic environment and a change in the intrinsic molecular makeup of aging cells. Early studies have demonstrated that RGCs require specific molecular signals for the stimulation of axon growth even without inhibitory molecules in the extrinsic environment, leading successive efforts to focus on uncovering the intrinsic signaling pathways that control axon extension during RGC development. Phosphatase and tensin homolog (PTEN), suppressor of cytokine signaling 3 (SOCS3), dual leucine zipper kinase, and krüppel-like factor (KLF) family members are some of the transcription factors and proteins that have been demonstrated to govern the intrinsic signaling pathways of axon regeneration (He and Jin, 2016). Whereas the molecular signatures that contribute to the differential axon regenerative potential between young and mature RGCs remain poorly understood, increasing evidence has revealed that microRNAs play a critical role in orchestrating the expression of transcription factors for axon growth in neurons of the central nervous system. A recent study has unveiled a previously unrecognized involvement of the miR-19a/PTEN axis in regulating the developmental decline of axon regenerative capacity in RGCs, highlighting the potential of microRNA-based therapeutics to rejuvenate aged RGCs and promote optic nerve regeneration (Mak et al., 2019).

Targeting microRNAs for neuroprotection and neuroregeneration: MicroRNAs (miRNAs) are short non-coding RNA molecules that function primarily as posttranscriptional regulators. To date, miRNA sequence repository miRBase (http://www.mirbase.org/) has identified a total of 38,589 hairpin precursor miRNAs (48,860 mature miRNAs) across 271 organisms. As the strong evolutionary conservation of miRNAs between species began to unfold with the advancement of miRNA gene expression technologies over the past two decades, an increasing number of studies have uncovered the contribution of miRNAs to the development, maintenance, and repair of the mammalian visual system. miRNAs are differentially expressed during normal development of the retina. In conditional knockout mouse models, retinas without Dicer, a key miRNA-processing enzyme, exhibit irregular laminar organization, aberrant RGC axon pathfinding, and defective developmental timing of neuronal differentiation (Reh and Hindges, 2018). Some miRNAs are expressed in specific layers of the retina. High-throughput next-generation sequencing has helped characterize various roles of miRNA in the retina, such as mediating light-dependent properties (Krol et al., 2010), rod photoreceptor survival (Sundermeier et al., 2014), and myelin repair after optic nerve injury (Wang et al., 2017). An advantage of targeting miRNAs for neuroprotection and neuroregeneration is their ability to bind onto more than one downstream target to enable simultaneous regulation of multiple pathways. For example, the miR-17/92 cluster has been predicted to target well-known regulators of axon regeneration such as PTEN, SOCS3, and several KLF family members (KLF2, KLF6, KLF7, KLF10, KLF12, KLF13, and KLF16). The ability of miRNA to regulate multiple downstream targets for axon regeneration and the fact that miRNA can be delivered locally to the inner retina make them an attractive therapeutic target for optic neuropathies.

Therapeutic potential of microRNAs for optic neuropathies: Therapeutic potential of miRNAs for neuroprotection and neuroregeneration has been shown promising in pre-clinical models of optic neuropathies. Glutamate neurotoxicity has been implicated in a variety of optic neuropathies including optic neuritis (Sucher et al., 1997). In a mouse model of autoimmune encephalomyelitis – a model of multiple sclerosis and optic neuritis – Morquette and colleagues demonstrated that adeno-associated virus (AAV)-mediated upregulation of miR-223 in RGCs via intravitreal injection protected RGC axons in the optic nerve from degeneration (Morquette et al., 2019). The observation of endogenous upregulation of miR-223 and miR-27a in neurons of the autoimmune encephalomyelitis model as well as neurons from post-mortem human cortical tissues of multiple sclerosis suggests a biological compensatory response for neuroprotection against glutamate neurotoxicity via binding onto downstream targets GluA2 and NR2B – major subunits of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate receptors, respectively. In an experimental glaucoma model induced by an intracameral microbeads injection (Peng et al., 2019), upregulating miR-200a levels via tail vein injection of miR-200a mimics was able to prevent RGC apoptosis and preserve ganglion cell layer thickness and ganglion cell counts in the retina. A plausible mechanism of neuroprotection is a direct binding of miR-200a onto fibroblast growth factor 7, in which its downregulation inactivates the mitogen-activated protein kinase signaling pathway and enhances RGC survival. In another experimental glaucoma mouse model induced by episcleral vein cauterization, downregulation of miR-149 was able to prevent RGC apoptosis, decrease ultrastructural damage in RGCs, and increase the expression levels of betacellulin, phosphatidylinositol 3-kinase, and serine/threonine-specific kinase – key regulators of neurogenesis, cell proliferation, cell growth, and cell viability in the retina (Nie et al., 2018). In a model of traumatic optic neuropathy via optic nerve crush, miR-204 prevented RGC death via upregulation of growth associated protein 43 (Wang et al., 2018). The collective findings of these pre-clinical studies demonstrate that intrinsic axon regenerative and neuroprotective mechanisms in adult RGCs can be reinstated by an alteration of specific miRNAs.

Why do RGCs lose axon regenerative capacity with age? While evidence of miRNA-mediated alleviation of RGC apoptosis in animal models of optic neuropathies demonstrates the therapeutic potential of miRNAs, whether miRNAs govern the intrinsic molecular events that lead to the developmental loss of axon regenerative capacity in RGCs remains elusive. As RGCs make up less than 5% of retinal cells, previous miRNA profiling studies using whole retinas can hardly inform the repertoire of miRNA expression in RGCs. In a recent study, we characterized the expression of miRNA profiles in purified rodent RGCs at different developmental ages (E21, P6, and P30) using microarray and found that 76 miRNAs were differentially expressed from embryonic to adult ages, which included five of six members of the miR-17/92 cluster (Mak et al., 2020). In this miRNA cluster, miR-19a was significantly downregulated in aged RGCs and predicted to target PTEN, a known inhibitor of optic nerve regeneration. The study identifies the miR-19a/PTEN axis to be a heterochronic marker for axon regenerative capacity in RGCs; developmental loss in ability to regenerate axons is connected to an intrinsic decline in miR-19a over time, relieving PTEN suppression which contributes to inhibition of axon regeneration (**Figure 1**). Increasing intracellular levels of miR-19a significantly enhanced axon outgrowth of postnatal rodent RGCs cultured in microfluidic chambers; whereas decreasing the endogenous levels of miR-19a showed significantly reduced axon outgrowth. Axon outgrowth was similarly augmented in RGCs purified from 69- and 75-year-old human donors supplemented with miR-19a. In a mouse model of optic nerve crush, replenishing miR-19a in adult RGCs via AAV-based delivery improved RGC survival and promoted axon regeneration. miRNA-mediated PTEN suppression, however, did not completely remove intracellular PTEN. The fact that miRNAs are fine tuners of gene expression and that the proportion of RGCs with upregulation of miR-19a depends on the transduction efficiency of AAV explains the weaker axon regeneration by miR-19a compared with PTEN knockout transgenic animals after optic nerve injury. Nevertheless, our findings underscore the potential of manipulating the levels of miRNA to rejuvenate the intrinsic capacity of axon regeneration for treatment of optic neuropathies.

Opportunities and challenges: Multiple signaling pathways have been implicated in promoting axon regeneration of RGCs. The ability of miRNAs to target multiple downstream targets makes it an attractive candidate for this task. However, the possible activation or suppression of undesired pathways may propagate off-target effects that can be equally detrimental. While there are several miRNA target prediction databases available, there are risks of false-positive or -negative predictions. Thus, experimental validation of individual

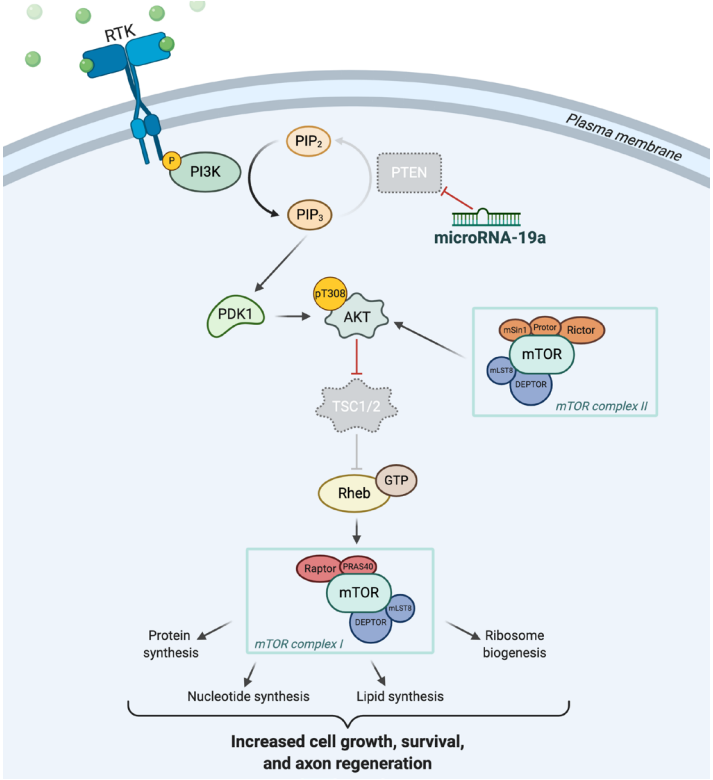

Figure 1 | **Illustration of the microRNA-19a/PTEN axis in regulation of axon regenerative capacity of retinal ganglion cells (RGCs).**
Supplementation of microRNA-19a suppresses elevated levels of endogenous PTEN, reactivating mTOR signaling and driving pro-axon regenerative pathways in mature RGCs. PTEN suppression allows PI3K, activated by RTK, to phosphorylate PIP_2 to PIP_3. Lipid second messenger PIP_3 in turn recruits downstream effector AKT to the membrane to be phosphorylated by PDK1 at threonine 308 (pT308). AKT-mediated phosphorylation of the TSC1/2 complex then relieves inhibition of Rheb, which leads to activation of the rapamycin-sensitive mTOR complex (mTORC1). Activated mTORC1 promotes cell growth, survival, and axon regeneration through upregulation of protein synthesis, nucleotide synthesis, lipid synthesis, and ribosome biogenesis. Of note, rapamycin-insensitive mTOR complex (mTORC2) is also able to activate AKT via phosphorylation at serine 473. Image created with BioRender.com. AKT: Serine/threonine-specific kinase (also known as PKB); mTOR: mammalian target of rapamycin; PDK1: 3-phosphoinositidedependent kinase; PI3K: phosphatidylinositol 3-kinase; PIP_2: phosphatidylinositol-4,5-bisphosphate; PIP_3: phosphatidylinositol-3,4,5-trisphosphate; PTEN: phosphatase and tensin homolog; RTK: receptor tyrosine kinase; TSC1: tuberous sclerosis 1 (also known as hamartin); TSC2: tuberous sclerosis 2 (also known as tuberin).

miRNA-mRNA interactions in the cell types of interest is key, as miRNA binding is dependent on cell type-specific RNA binding factors, mRNA folding, rates of translation, and competing transcripts. Furthermore, although imperfect binding of miRNA onto unknown mRNA may cause toxicity, new approaches are being explored by simultaneously administering miRNA mimics and inhibitors to silence and activate specific pathways, respectively, to ameliorate toxic effects. Lastly, endogenous function of RISC, the multiprotein complex that recognizes and cleaves complementary mRNA, in the target cell type must also be considered. In cells where RISC is downregulated, delivery of miRNAs may not be functionally useful.

Whereas miRNA-based therapy for optic neuropathies has yet to enter Phase I clinical trial, the safety of miRNA-based therapeutics has been demonstrated for treatment of systemic neurodegenerative diseases including Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis; a number of miRNA mimics and anti-miRNA are being evaluated in Phase II/III trials (Roberts et al., 2020). Of note, in comparison with intrathecal or systemic routes of administration for systemic neurodegenerative diseases, an intraocular mode of delivery of miRNA-based therapeutics for optic neuropathies has the advantage of providing greater bioavailability of miRNAs to target cells with less off-target side effects by bypassing common barriers that require penetration of the blood retinal barrier and overcoming extrinsic inhibitory environments before reaching the visual system. Whereas an AAV-mediated delivery of genes to RGCs via intravitreal injection has been a demonstrably safe and effective method to date, a variation in treatment responses could still arise due to variable viral transduction efficiencies and endogenous levels of downstream target genes between patients. Further work on increasing AAV transduction efficiency by capsid modification, enhancing viral load, and regulating host immune responses, and developing new technologies to detect target gene levels in patients will contribute to improving the efficacy of potential ocular gene therapies.

One major challenge in the evaluation of treatment effect for any neuroprotective or neuroregenerative therapy is being able to discern the integrity of RGCs and their axons with reliability and precision. Many forms of optic neuropathies, such as glaucoma, are slowly progressive; it may take years to detect a neuroprotective effect in clinical trials. With the advancement of digital imaging technologies of the retina, it is now feasible to visualize individual axonal fiber bundles and their loss over the retina within seconds in the clinic using optical coherece tomography (our unpublished data). The deployment of more precise and reliable biomarkers for the assessment of RGC axons holds promise to expedite clinical trial research and approval of neuroprotective or neuroregenerative therapeutics for optic neuropathies.

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References

He Z, Jin Y (2016) Intrinsic control of axon regeneration. Neuron90:437-451.
Krol J, Busskamp V, Markiewicz I, Stadler MB, Ribi S, Richter J, Duebel J, Bicker S, Fehling HJ, Schübeler D, Oertner TG, Schratt G, Bibel M, Roska B, Filipowicz W (2010) Characterizing light-regulated retinal microRNAs reveals rapid turnover as a common property of neuronal microRNAs. Cell 141:618-631.
Mak HK, Yung JSY, Weinreb RN, Ng SH, Cao X, Ho TYC, Ng TK, Chu WK, Yung WH, Choy KW, Wang CC, Lee TL, Leung CK (2020) MicroRNA-19a-PTEN axis is involved in the developmental decline of axon regenerative capacity in retinal ganglion cells. Mol Ther Nucleic Acids 21:251-263.
Morquette B, Juźwik CA, Drake SS, Charabati M, Zhang Y, Lécuyer MA, Galloway DA, Dumas A, de Faria Junior O, Paradis-Isler N, Bueno M, Rambaldi I, Zandee S, Moore C, Bar-Or A, Vallières L, Prat A, Fournier AE (2019) MicroRNA-223 protects neurons from degeneration in experimental autoimmune encephalomyelitis. Brain 142:2979-2995.
Nie XG, Fan DS, Huang YX, He YY, Dong BL, Gao F (2018) Downregulation of microRNA-149 in retinal ganglion cells suppresses apoptosis through activation of the PI3K/Akt signaling pathway in mice with glaucoma. Am J Physiol Cell Physiol 315:C839-849.
Peng H, Sun YB, Hao JL, Lu CW, Bi MC, Song E (2019) Neuroprotective effects of overexpressed microRNA-200a on activation of glaucoma-related retinal glial cells and apoptosis of ganglion cells via downregulating FGF7-mediated MAPK signaling pathway. Cell Signal 54:179-190.
Reh TA, Hindges R (2018) MicroRNAs in retinal development. Annu Rev Vis Sci 4:25-44.
Roberts TC, Langer R, Wood MJA (2020) Advances in oligonucleotide drug delivery. Nat Rev Drug Discov 19:673-694.
Sucher NJ, Lipton SA, Dreyer EB (1997) Molecular basis of glutamate toxicity in retinal ganglion cells. Vision Res 37:3483-3493.
Sundermeier TR, Zhang N, Vinberg F, Mustafi D, Kohno H, Golczak M, Bai X, Maeda A, Kefalov VJ, Palczewski K (2014) DICER1 is essential for survival of postmitotic rod photoreceptor cells in mice. FASEB J 28:3780-3791.
Wang H, Moyano AL, Ma Z, Deng Y, Lin Y, Zhao C, Zhang L, Jiang M, He X, Ma Z, Lu F, Xin M, Zhou W, Yoon SO, Bongarzone ER, Lu QR (2017) miR-219 cooperates with miR-338 in myelination and promotes myelin repair in the CNS. Dev Cell 40:566-582. e5.
Wang N, Yang W, Xiao T, Miao Z, Luo W, You Z, Li G (2018) Possible role of miR-204 in optic nerve injury through the regulation of GAP-43. Mol Med Rep 17:3891-3897.

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