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 connects 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 regeneration capacity of RGCs is one contributor to 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 kruppel-like factor 15 (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 and proteins 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/14/) 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, the expression of miRNAs has been extensively studied in the last two decades, with the number of studies that 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 also differentially expressed in 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 the 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., 1996). MicroRNA-200a onto fibroblast growth factor 7, in which D-aspartate receptors, respectively. In an experimental glaucoma mouse model induced by an intracamerical 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 intracamerical microbeads injection, miR-200a activated axon regeneration. miR-200a onto downstream targets GluA2 and NR2B promoted axon regeneration. miRNA-mediated AAV-based delivery improved RGC survival and promoted axon regeneration. miRNA-mediated PTEN suppression, however, did not completely remove intracellular PTEN. The effect of miRNAs is fine-tuned of gene expression and the fact 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 knockdown. Transgenic animal models of optic neuropathy demonstrate the potential of manipulating the levels of miRNA to rejuvenate the intrinsic capacity of axon regeneration for treatment of optic neuropathies.

Why do RGCs lose axon regenerative capacity with age? While evidence has suggested that the developmental decline of axon regenerative capacity is due to a change in the extrinsic environment and a change in the intrinsic 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 kruppel-like factor 15 (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 and proteins 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).

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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 the number of target prediction databases available, there are risks of false-positive or negative predictions. Thus, experimental validation of individual
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 mTORC1, to promote 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.

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