Relationship between the Pathogenesis of Glaucoma and miRNA

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Abstract
Small RNA (microRNA or miRNA) is a kind of small noncoding single-stranded RNA that regulates complementary mRNA at the posttranscriptional level in eukaryotic organisms. As important regulatory factors, miRNAs play an important role in the occurrence and development of glaucoma and widely participate in regulating biological processes of glaucoma-related genes. This article reviews the connection between the aqueous humor, trabecular meshwork, the apoptosis of retinal ganglion cells, and miRNA.

Introduction
Small RNAs (microRNAs or miRNAs), which are 21-to 23-nucleotide-long single-stranded RNAs that regulate about 30% of encoding genes, can interact with the target mRNA 3′-UTR and allow the degradation of mRNA or translation inhibition [1]. One kind of miRNA could target a variety of mRNAs. miRNA is an important composition regulation of eukaryotic gene expression [2, 3] and takes part in a diverse array of biological functions, including cell differentiation, proliferation, apoptosis, individual development, and the body’s metabolism [4]. Indeed, miRNAs are observed to be involved in the pathological processes of numerous diseases, such as the occurrence of tumor [5] and obesity [6]. miRNAs can also be used as noninvasive biomarkers for the diagnosis of numerous diseases [7]. Further, Lee et al. [8] discovered the first miRNA upon studying the development of nematode, and more than 2,000 kinds of miRNAs have been found in organisms to date. Lagos-Quintana [9] identified 7 kinds of miRNAs from adult rat eyes for the first time in 2003; thus, the role of miRNA came to be gradually known.

Glaucoma is a neurodegenerative disease characterized by the progressive loss of retinal ganglion cells (RGCs) and optic nerve axons, and it is accompanied with loss of visual field sensitivity. In addition, glaucoma leads to irreversible visual loss and affects approximately 60 million people worldwide [10]. However, its pathogenesis remains unclear. Moreover, a large number of domestic and foreign studies have shown that glaucoma is a multifactorial disease. Therefore, the molecular genetics of glaucoma have become a hotspot in the field of ophthalmology. miRNA is linked with maintaining the...
balance of the aqueous humor, the change in the trabecular meshwork, and the apoptosis of RGCs. This article reviews the relationship between the pathogenesis of glaucoma and miRNA, providing a new method for the further study of the possible role of miRNA in glaucoma.

The Relationship between miRNA and the Aqueous Humor

The aqueous humor fills the anterior and posterior chambers of the eyeball. It is mainly formed by ciliary epithelial cells, and it is always in a dynamic balance. This balance can maintain the intraocular pressure, providing the cornea, lens, and vitreous body with nutrition while removing tissue metabolites. In addition, the main risk factor for glaucoma is high intraocular pressure, and elevated intraocular pressure is relevant to the disorder in the dynamic balance between the generation and discharge of aqueous humor [11–13]. Moreover, the main methods of treating glaucoma involve the reduction of aqueous humor production and the promotion of aqueous humor drainage, thereby relieving the intraocular pressure [14].

A large number of studies have shown that miRNA is not found only within the cell but also in the outside of the cell. The extracellular miRNAs exist in several ways, such as in the form of the exosome [15, 16], apoptosis body [17], and protein/miRNA complexes [18]. Studies have found that miRNA exists in numerous bodily fluids (e.g., serum, saliva, urine, and tears) [19], and some miRNAs are uniquely present in specific bodily fluids, such as miRNA-224 in plasma, miRNA-637 in tears, and miRNA-193b in breast milk [19]. Some researchers collected aqueous humor from cataract patients in accordance with the inclusion criteria. They detected 264 miRNAs using real-time PCR and 110 species of miRNAs in the aqueous humor, of which the most abundant 5 were miRNA-202, miRNA-193b, miRNA-135a, miRNA-365, and miRNA-376a. These miRNAs maybe maintain the shape of the anterior chamber and the pressure of the aqueous humor by regulating the target genes of the organizations related to the anterior chamber, such as the trabecular meshwork. In addition, 20 kinds of miRNAs were compared in the aqueous humor, revealing that 17 kinds of miRNAs may exist only in the aqueous humor, which demonstrated the tissue specificity [20]. Additionally, some researchers collected aqueous humor through the anterior chamber paracentesis and evaluated the total RNA concentration (14–85 ng/mL) and length (<200 nt) in the aqueous humor for the first time [21]. Based on their findings, in glaucoma patients, 11 kinds of miRNAs were significantly upregulated, and 18 kinds of miRNAs were significantly downregulated. The differences among the methods of separating and detecting miRNAs as well as analyses of biological information can account for the differences found among various experimental results. These studies suggested that miRNA in the aqueous humor may serve as a biomarker for glaucoma, allowing a more convenient glaucoma diagnosis.

The Relationship between miRNA and the Trabecular Meshwork

The trabecular meshwork is a major organization that affects aqueous humor drainage. The balance of the aqueous humor can be affected by the deposition of the extracellular matrix in the trabecular meshwork, the contraction of trabecular meshwork cells, and the abnormalities in the trabecular meshwork structure.

Besides, with the rapid developments of molecular biology and the successful implementation of the human genome project, the studies on genes associated with glaucoma have also made immense progress. A total of 962 kinds of nonhousekeeping genes in the trabecular meshwork tissue were measured by Target Scan prediction software. It was found that miRNA can influence changes in the trabecular meshwork by regulating gene expression in trabecular meshwork tissue [22]. Researchers transfected trabecular meshwork cells with an miRNA-29b mimic and found that the expression of genes was associated with the deposition and reform of the extracellular matrix [23]. One study found that miRNA-204 could not only reduce the expression of FOXCL, a transcription factor that can affect eye development and be expressed in the trabecular meshwork, but also downregulate the expression of FOXCL target genes (e.g., CLOCK, PLEKHG5, ITGβ1, and MEIS2) in the trabecular meshwork [24]. The study suggested that a complex regulatory system could affect the occurrence and development of glaucoma [24].

The Relationship between miRNA and the Extracellular Matrix in the Trabecular Meshwork

The acknowledged mechanism is aqueous humor outflow retardation for the excessive accumulation of the extracellular matrix (e.g., collagen, fibronectin, and laminin) in the trabecular meshwork, which leads to elevated
intraocular pressure. Numerous growth factors, such as transforming growth factor (TGF-β), connective tissue growth factor, and basic fibroblast growth factor, affect the changes of the extracellular matrix in the trabecular meshwork. The TGF-β subfamily of cytokines includes a series of multifunctional proteins [25] and participates in many biological processes such as inflammation, wound healing, the generation and accumulation of extracellular matrix, and so on [26]. The relationship between TGF-β and fibrosis is reflected in many diseases [27]. Studies showed that trabecular meshwork cells synthesized and secreted TGF-β, and TGF-β influenced the synthesis of the extracellular matrix in primary open-angle glaucoma by regulating connective tissue growth factor [28]. In addition, TGF-β might contribute to changing the structure of the extracellular matrix in the trabecular meshwork. Studies found that TGF-β2 could induce the cultured trabecular meshwork cells secreting the bone morphogenetic protein [29], and bone morphogenetic protein was known to increase the crosslinking of extracellular matrix proteins by activating the LOX enzyme. These findings suggested that TGF-β2 could accelerate the maturation of extracellular matrix proteins, which increased the stiffness of the trabecular meshwork and aqueous outflow resistance [29].

Other researches have shown that miRNA could control TGF-β, which affected the metabolism of extracellular matrix in the trabecular meshwork. miRNA-24 was able to negatively regulate the expression of the anthropogenic transformation of protease that activated TGF-β by targeting the anthropogenic transformation of protease (FURIN), and the overexpression of miRNA-24 could downregulate the expression of TGF-β [30]. By cultivating the trabecular meshwork cells with recombinant TGF-β, researchers found that the miRNA-29 family was expressed in the trabecular meshwork cells by using real-time PCR, and the overexpression of the miRNA-29 family could inhibit extracellular matrix protein synthesis (SPARC, collagen I, collagen IV, and laminin) [31]. In addition, the miRNA-29 family might regulate the effect of TGF-β on the extracellular matrix [32]. Our research team showed that miRNA-483-3p had an inhibitory effect on extracellular matrix production in the human trabecular meshwork cells through downregulating Smad4 [33], which targeted TGF-β/bone morphogenetic protein [34] and laminin [35, 36]. As a consequence, we may draw the conclusion that miRNA serves as a potential therapeutic target in influencing the development of glaucoma by regulating TGF-β, which affects the extracellular matrix.

The Relationship between miRNA and the Contraction of Trabecular Meshwork Cells

The trabecular meshwork is one of the important structures in the outer course of the aqueous humor circulation. Studies have shown that the shrinkage of silks takes place in the trabecular meshwork, which is consistent with the function of the trabecular meshwork [37, 38]. When the trabecular meshwork cells relax, the filtration area of aqueous humor is increased, and the intraocular pressure is reduced [39–41]. On the contrary, the contraction of trabecular meshwork cells can bridge the gap between trabecular meshwork cells, resulting in a decline in the permeability of the trabecular meshwork and affecting the discharge of aqueous humor [42, 43]. Researchers found that miRNA-200c could inhibit the expression of genes (ZEB1, ZEB2, FHOD1, LPAR1/EDG2, ETAR, and RHOA) related to the contraction of trabecular meshwork cells and discovered that the contraction of collagen was inhibited and the traction of trabecular meshwork cells was reduced when rat eyes were transfected with miRNA-200c [44]. In addition, intraocular pressure is reduced after an intraocular injection of miRNA-200c, which might be a novel target for the treatment of glaucoma [44]. Besides, researchers found that miRNA-450 could influence the shrinkage of trabecular meshwork cells by changing the MyoD family proteins [45]. Prompting the miRNA influenced the outflow of the aqueous humor through changing the structure of the smooth muscle tissue, which provided a new method for the drug therapy of glaucoma.

The Relationship between miRNA and the Aging Trabecular Meshwork Cells

Normal trabecular meshwork tissue is essential for maintaining normal anterior chamber pressure, and the morphology of trabecular meshwork cells can be transformed in pathological conditions of high intraocular pressure. The aging of trabecular meshwork cells may increase the aqueous humor outflow channel resistance, and oxidative stress plays an important role in the pathogenesis of the aging of trabecular meshwork cells [46]. The expression of miRNA in the trabecular meshwork would be changed under the condition of oxidative stress. Li et al. showed that 14 kinds of miRNAs were downregulated and 3 kinds of miRNAs of the trabecular meshwork cells were upregulated with the treatment of H2O2 [47]. Some studies revealed that the upregulation of miRNA-146a can downregulate the expression of genes related to inflammation and cell senescence [48]. Moreover, oxidative stress could also influence intraocular pressure.
pressure by regulating the apoptosis of trabecular meshwork cells [49]. Studies found that the sensitivity of apoptosis and the number of trabecular meshwork cells also increased after the trabecular meshwork cells were transfected with miRNA-204, which hinted that miRNA-204 plays an important role in the regulation of trabecular meshwork cell function [50]. After trabecular meshwork cells had dealt with H2O2, miRNA-183 could decrease the expression of laminin, gel, and type I collagen through targeting ITGβ1 lacking the 3′-UTR [51]. The conclusion may be drawn that miRNA may regulate the apoptosis of the trabecular meshwork, which influences the intraocular pressure of the organization.

The Relationship between miRNA and the Apoptosis of RGCs Caused by Glaucoma Damage

Glaucoma has the usual pathological outcome of common retinal optic nerve damage. The apoptosis of RGCs is the main cause of optic nerve damage in glaucoma, and numerous factors are involved in this process [52, 53]. In addition, miRNAs exist in the retina [54], and miRNAs are expressed in the retina of eyes with advanced glaucomatous damage [55]. A comprehensive understanding of the mechanisms of RGC apoptosis is highly important in treating glaucoma. Researchers discovered that the up-regulation of miRNA-96 could decrease the activity of RGCs, and this effect was achieved through the activation of caspase. The role of miRNA-96 in RGCs disappeared when the caspase-2 gene was silenced, which indicated that miRNA-96 was likely to affect the survival and apoptosis of RGCs through interaction with caspase-2 [56]. The other mode of RGC apoptosis is to initiate the intrinsic apoptosis pathway which disrupts the mitochondrial function [57]. Kong et al. [58] found that the downregulation of miRNA-100 mediated by lentivirus might reduce the apoptosis of RGCs and promote nerve growth through the phosphorylation pathway. In conclusion, miRNAs may be used to reduce the apoptosis of RGCs, which indirectly increases the quality of life of patients with glaucoma.

Conclusion

miRNA is a hotspot in the field of medicine and has been widely used in the diagnosis and treatment of diseases. Hundreds of miRNAs have been found in eye tissues to date. miRNA is associated with the development of many ocular diseases, such as retinoblastoma [59], autoimmune uveoretinitis [60], and familial keratoconus [61]. Nevertheless, studies of miRNA in glaucoma are still in the primary stage. Confirmation of the specific expression of miRNA in glaucoma and its target genes may become the focus of future research, as well as how miRNA regulates biological behavior related to glaucoma. In the near future, miRNA might become a novel noninvasive biomarker in the early diagnosis, evaluation of progression, and prognosis of glaucoma, as well as in the development of novel therapeutic strategies in treating patients with glaucoma and in giving us a new thinking direction of how to reverse or slow down the progression of glaucoma.

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Disclosure Statement

The authors declare no conflict of interest.

References

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