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MicroRNA in the pathogenesis of glaucoma

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HIGHLIGHTS

MicroRNAs are involved in the pathogenesis of glaucoma and may be potential biomarkers and therapeutic target in glaucoma.

ABSTRACT

MicroRNAs are short ribonucleic acid molecules that regulate gene expression. The involvement of various types of microRNAs in the pathogenesis of glaucoma has been proved. Most of them affect trabecular meshwork in the anterior chamber angle, causing excessive deposition of extracellular matrix and blockage of the aqueous humor outflow. MicroRNAs affect the contractility of the trabecular meshwork cells, decreasing its permeability and increasing intraocular pressure. They participate in the regulation of apoptosis of trabecular meshwork cells and retinal ganglion cells. MicroRNAs may be potential biomarkers for glaucoma and, in the future, a target for gene therapy.

Key words: microRNA, glaucoma, trabecular meshwork, oxidative stress

INTRODUCTION

MicroRNA (miRNA) is a short ribonucleic acid molecule, made up of 21–23 nucleotides. According to current knowledge, genes encoding miRNAs constitute approx. 2% of the human genome. Their function is to regulate gene expression at the post-transcriptional level. The process may involve up to 30% of structural genes. Such a significant influence on the genotype results from the fact that one miRNA molecule can affect several hundred genes. Gene expression is regulated by interference, i.e. silencing or turning them off by blocking mRNA translation [1]. MicroRNAs can act as both suppressor genes and oncogenes [2]. Suppressor genes, also known as anti-oncogenes, control the processes of cell growth and division. They maintain their appropriate number in the body by inhibiting proliferation or activating apoptosis – the process of programmed cell death. Oncogenes cause uncontrolled multiplication and survival of cells [3].

MICRORNA IN DISEASES

miRNA molecules were discovered in 1993 in the *Caenorhabditis elegans* nematode [4]. This event initiated intensive research into their role in physiological processes as well as in pathogenesis of various diseases.

Aging is an example of a physiological process involving miRNA. It consists of a set of dynamic changes at the structural and functional level of the organism, taking place under the influence of time and environmental factors [5]. MiR-17-5p, which regulates the cell cycle, proliferation and apoptosis, has been shown to play an important role in this phenomenon. In experimental models of aging, the expression of miR-17-5p decreases, while it is increased in the blood of cancer patients, maintaining the survival of cancer cells [6]. MicroRNA is involved in the development of many other age-related diseases, including coronary artery disease, stroke, myocardial infarction, hypertension, diabetes and osteoporosis [7]. Changes in miRNA expression have also been demonstrated in progressive neurodegenerative diseases, sharing the common feature of loss of nerve cells. These conditions include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, Huntington's disease, and glaucoma. MicroRNAs associated with neurodegeneration include the miR-29 family, miR-21-5p, miR-132-3p, miR-124-3p, miR-146a-5p, miR-155-5p, miR-223-3p, and miR-9-5p [8].

Since the identification of the first miRNA in eye tissues in 2003 by Lagos-Quintana et al., miRNA disturbances have been confirmed in numerous eye diseases, including glaucoma, cataract, age-related macular degeneration, diabetic retinopathy, Fuchs dystrophy, as well as in neoplasms such as retinoblastoma and uveal melanoma [9, 10]. MicroRNA dysregulation has also been demonstrated in systemic diseases with ocular symptoms, such as Graves' disease, Behçet's disease, Vogt-Koyanagi-Harada syndrome and Sjögren's syndrome [11–14].

The participation of miRNA in the development of such diverse diseases confirms regulation of many genes in the body by these molecules. These genes are involved in processes of cell differentiation and growth, proliferation, apoptosis, and are also responsible for cellular metabolism, signaling and the function of stem cells [15].

Research on miRNAs is important for understanding of many complex metabolic pathways leading to visual impairment. They create a possibility of using miRNAs as molecular biomarkers with predictive, diagnostic and prognostic value. They also mark a new direction of therapy in ophthalmology, which may become common in the future.

GLAUCOMA

Glaucoma is one of the most important causes of irreversible loss of vision. It is estimated that about 80 million people in the world suffer from glaucoma, and 11 million of them suffer from binocular blindness [16]. The pathogenesis of glaucoma is not fully understood. A relationship between increased intraocular pressure (IOP) and apoptosis of retinal ganglion cells has been proven. However, there are also cases of glaucoma in which IOP values are within their normal range and yet neuropathy progresses. This shows that other mechanisms that damage the optic nerve exist. They may result from genetic conditions and vascular disorders [17]. The value of IOP depends on the balance between production of aqueous humour by the ciliary body and its outflow via three independent pathways – the trabecular meshwork, the uveoscleral pathway, and the iris [18].

MICRORNA IN GLAUCOMA

MicroRNA regulates numerous physiological processes in the eyeball – it maintains the balance of aqueous humour, proper functioning of trabecular meshwork and retinal ganglion cells. However, due to various factors, e.g. oxidative stress, mechanical stress, or ischemia, miRNA can induce pathological changes that contribute to development of glaucoma. Target miRNA structures are found in both the anterior and posterior segments of the eyeball. Moreover, a miRNA-involving interaction between these segments has been demonstrated.

Depending on the criterion, there are different types of glaucoma. Studies conducted in patients with primary open-angle glaucoma (POAG) and pseudoexfoliation glaucoma (PEXG) show that different types are characterized by different miRNA expression. In one of the studies on POAG, there was a significant upregulation of miR-518d and miR-143 and a downregulation of miR-660 compared

to the control group [19]. In another trial, three different miRNAs were identified between subjects with POAG and the control group (miR-125b-5p, miR-302d-3p, miR-451a), five different miRNAs between subjects with PEXG and the control group (miR-122-5p, miR-3144-3p, miR-320a, miR-320e, miR-630) and one different miRNA between POAG and PEXG subjects (miR-302d-3p). It was proved that miR-122-5p regulated three genes related to glaucoma: *OPTN*, *TMCO1* and *TGF-ß1* (*transforming growth factor ß1*) [20]. More research is needed to determine the exact function of other miRNAs in the pathogenesis of glaucoma, and to identify potential biomarkers in different types of the disease.

TRABECULAR MESHWORK

Trabecular meshwork, which connects the scleral spur and Schwalbe's line, and covers the interior of Schlemm's canal, have the greatest share in the aqueous humour outflow from the anterior chamber. Trabecular meshwork is an avascular connective tissue demonstrating a complex architectural structure. There are three filtering layers:

- uveal meshwork, made of collagen and elastin plates, arranged loosely and covered with trabecular meshwork cells
- corneoscleral meshwork, containing packets of perforated plates with trabecular meshwork cells
- endothelial layer.

The aqueous humour flows from the anterior chamber through the uveal and corneoscleral meshwork, which trap cellular debris and reactive oxygen species (ROS) before they reach the endothelial layer. The trabecular meshwork endothelial layer generates resistance, limiting the aqueous humour outflow to the lumen of Schlemm's canal and the venous system [21].

Trabecular meshwork cells are surrounded by an extracellular matrix (ECM) that fills the spaces between corneoscleral meshwork plates and the inner wall of Schlemm's canal. ECM consists of collagen, elastin, glycosaminoglycans, proteoglycans and glycoproteins. The extracellular matrix has the greatest impact in generating resistance to the aqueous humour outflow. Exact mechanisms of this process are unknown, but it is known that the basal membrane, on which the cells of the inner wall of Schlemm's canal lie, plays an important role. The basal membrane contains type I and IV collagen, laminin and integrin. Interaction between laminin and integrin ensures adhesion of cells of Schlemm's canal to the basal membrane and ECM. This maintains continuity of the barrier, and allows maintenance of an appropriate resistance of aqueous humour outflow depending on the IOP. It was confirmed that ECM can undergo remodeling depending on the condition of trabecular meshwork cells. Their mechanical stretching under the influence of increased IOP increases the activity of metalloproteinases in ECM, affecting expression of numerous ECM proteins. It was shown that inhibition of metalloproteinases reduced aqueous humour outflow, while high metalloproteinase activity increased aqueous humour outflow. Trabecular meshwork cells also secrete tenascin-C and α-smooth muscle actin into ECM. In healthy tissues, their activity is low, but increases in the trabecular meshwork in patients with glaucoma. This leads to excessive accumulation of collagen and other proteins in ECM. Formation of thickened plates by elastic fibers has also been confirmed. As a result, ECM becomes denser and stiffer and increases the resistance of aqueous humour outflow, resulting in increased IOP [22]. In the experimental model of steroid glaucoma, it was shown that dexamethasone increased the synthesis and deposition of ECM proteins. Fibronectin – the main regulator of the ECM structure – participated in induction of stress in the endoplasmic reticulum of trabecular meshwork cells, which could contribute to trabecular meshwork dysfunction and increased IOP [23].

OXIDATIVE STRESS

The disturbed balance between ROS and cellular antioxidants is known as oxidative stress. Reactive oxygen species are highly reactive derivatives of molecular oxygen. At low concentrations, they are essential in many metabolic processes of the body, participate in proliferation, apoptosis, signal transmission to cells and gene expression [24]. The main source of ROS is oxidative phosphorylation in the mitochondrial respiratory chain, which provides over 90% of energy for the body in the form of ATP (adenosine triphosphate). Reactive oxygen species are also produced with the participation of nicotinamide adenine dinucleotide oxidase (NADPH) during rapid oxygen transformations in phagocytes, which enables the fight against bacteria and viruses in the body. Many other oxidases are involved in production of ROS, including those in the endoplasmic reticulum and peroxisomes [25]. To maintain homeostasis in the body, excessive ROS have to be neutralized by antioxidants. The most important cellular antioxidants are: cytoplasmic superoxide dismutase containing copper and zinc, mitochondrial superoxide dismutase containing manganese, catalase, glutathione peroxidase and glutathione reductase. Oxidative stress can be triggered by a number of overlapping factors, including excessive ROS production, mitochondrial dysfunction, and impaired antioxidant systems. Moreover, presence of oxidative stress additionally increases the production of ROS. Excessive production of ROS occurs during physiological ageing, as well as in acute or chronic pathological changes. As a result, DNA, structural and enzymatic proteins and lipids are damaged. Mitochon-

drial DNA (mtDNA) is particularly susceptible to oxidative damage because it is close to the inner mitochondrial membrane and has less efficient repair systems. Mitochondrial DNA mutations cause disturbances in the respiratory chain and further uncontrolled production of ROS [24].

TRABECULAR MESHWORK AND OXIDATIVE STRESS

Trabecular meshwork is the structure most exposed to oxidative stress in the anterior segment of the eye, as it is not directly exposed to light and has a weaker antioxidant defense [26]. In patients with glaucoma, a significantly lower antioxidant potential was demonstrated compared to the control group [27]. Reactive oxygen species produced by metabolic and inflammatory processes are present in the aqueous humour that comes into contact with trabecular meshwork. When homeostasis is disturbed and oxidative stress arises, damage to mtDNA, proteins and lipids of trabecular meshwork cells occurs. This leads to their apoptosis or trabecular meshwork structural changes, especially in ECM, which decreases the aqueous humour outflow and results in increased IOP [28].

MICRORNA IN TRABECULAR MESHWORK

Trabecular meshwork is located in the iridocorneal angle and is responsible for the aqueous humour outflow from the anterior chamber of the eye into epidural veins. Increased synthesis and deposition of ECM proteins leads to trabecular meshwork dysfunction. Studies have shown that ECM disturbances are the main factor inhibiting the aqueous humour outflow and causing an increase in IOP [29].

Different types of miRNAs influence functions of ECM. One study showed that miR-183 expression increased under conditions of oxidative stress induced by H_2O_2. Oxidative stress is used to build an experimental model of glaucoma because it is induced by a decrease in oxygen flow and a hypoxic state of trabecular meshwork cells due to a reduction in blood flow velocity in glaucoma patients. miR-183 molecules inhibit activity of the receptor (β_1 integrin) involved in cell interactions with ECM proteins. Overexpression of miR-183 disrupts trabecular meshwork physiology, leading to development of glaucoma [30].

MicroRNA-29b negatively regulates expression of numerous genes involved in ECM synthesis and deposition in trabecular meshwork cells. Expression of miR-29b decreases under chronic oxidative stress, resulting in increased production of fibrous proteins (collagen, fibrillin) and non-fibrous proteins (laminin), which are components of ECM. This increases density and stiffness of the ECM and decreases aqueous humour outflow. The decrease in miR-29b expression also activates proteins responsible for ECM remodeling. As a result of these processes, ECM homeostasis becomes disturbed, which promotes loss of trabecular meshwork cells and leads to an increase in IOP [31].

TGF-β_2 has been shown to be significantly elevated in aqueous humour of patients with POAG. Transforming growth factor β_2 reduces miR-29b expression and increases miR-29a, which stimulates ECM synthesis and deposition [32, 33].

Another example of a miRNA that plays an important role in the pathomechanism of glaucoma is miR-144-3p. Its expression is lower in patients with POAG compared to the control group. MicroRNA-144-3p inhibits the multifunctional ECM protein – fibronectin – which plays a structural role and participates in intracellular signal transmission. Increased fibronectin activity promotes ECM deposition and trabecular meshwork blockade [34].

Trabecular meshwork is sensitive to mechanical forces. Fluctuating IOP causes its deformation – stretching with increased IOP and shrinking with decreased IOP. This leads to changes in morphology of trabecular meshwork cells and release of TGF-β_1, which increases the amount of collagen in ECM, and thus increases resistance to aqueous humour outflow.

Transforming growth factor β_1 is converted to its active form by the enzyme furin. This process is inhibited by miR-24, expression of which increases during chronic mechanical stress. It has also been shown that TGF-β_1 increases expression of miR-24 [35].

MicroRNAs can influence trabecular meshwork cells that play an important role in regulating IOP. MicroRNA-200c administered to the anterior chamber of the eye caused a significant decrease in IOP, while its inhibition by the adenoviral vector resulted in increased IOP. High expression of miR-200c in trabecular meshwork cells and its effect on contractility of these cells was demonstrated. Trabecular meshwork cells demonstrate properties similar to those of smooth muscles. They can relax or contract in response to biological or pharmacological factors. Cell contraction reduces intercellular spaces and thus trabecular meshwork permeability and aqueous humour outflow; their relaxation has the opposite effect. MicroRNA-200c has a relaxing effect on trabecular meshwork cells by down-regulating various regulatory proteins, receptors and enzymes, leading to a decrease in IOP [36].

Other miRNAs are known to affect contractility of trabecular meshwork cells. It has been shown that expression of miR-143 and miR-145 in trabecular meshwork is about 100–1000 times higher than, for example, in choroidal endothelial cells. Experimental removal of these miRNAs resulted in an approx. 19% decrease in IOP, being a consequence of approximately double increase in aqueous

humour outflow. These molecules regulate contractility of trabecular meshwork cells by influencing contractile proteins – actin and myosin. They can also affect contractility of smooth muscle cells in vessels. In the experimental model, removal of miR-143 and miR-145 resulted in an approx. 19% decrease in systolic blood pressure [37]. Lowering systemic blood pressure may correlate with reduced IOP, although the exact mechanism of this is not known [38]. It is presumed that circulating hormones, such as vasopressin, may be involved. Their level changes in patients with arterial hypertension [39].

In the analysis of miR-17-5p expression, oxidative stress induced by H_2O_2 was used. It showed that H_2O_2 decreased miR-17-5p expression in trabecular meshwork cells, which inhibited their proliferation and stimulated apoptosis. A specific suppressor protein is involved in this mechanism. An increase in miR-17-5p expression stimulates anti-apoptotic proteins Bcl-2 (*B-cell lymphoma-2*) and Bcl-xL (*B-cell lymphoma-extra-large*) and inhibits the pro-apoptotic protein Bax (*B-cell lymphoma-associated X protein*), protecting trabecular meshwork cells from apoptosis [40].

FACTORS DAMAGING RETINAL GANGLION CELLS

Oxidative stress

Reactive oxygen species can have a direct intracellular cytotoxic effect in retinal ganglion cells, damaging mainly mitochondria, or they can circulate in extracellular space and lead to disruption of previously healthy retinal ganglion cells. Loss of retinal ganglion cells occurs through apoptosis or autophagy. Autophagy is the process of breaking down and recycling cell components. It occurs in lysosomes, and is essential for maintenance of homeostasis and cell survival. Additionally, it is intensified by oxidative stress. Reactive oxygen species are also formed in glial cells that have a protective and nourishing function for the retinal ganglion cells. Increase in oxidative damage in the glial cells, combined with the failure of their repair systems, causes glial dysfunction and impaired protection of retinal ganglion cells against damage [41].

Mechanical stress

Important factors of glaucomatous neurodegeneration include mechanical pressure due to elevated IOP, which interferes with axonal transport and neurotrophin delivery [42]. Neurotrophins are proteins synthesized and secreted by nerve cells. They participate in synaptic formation and regulate differentiation, maturation and survival of neurons [43]. Insufficient amount of neurotrophins contributes to the apoptosis of the retinal ganglion cells [42].

Ischemia

Reduced blood flow in the retina, causing oxygen and nutrient deficiencies, also leads to damage of retinal ganglion cells. In addition, it has been shown that reperfusion, that is, restoration of blood flow after ischemia, causes oxidative and inflammatory cell damage. Reduction of blood flow in the retina occurs not only with increased IOP, but also in glaucoma with normal pressure [28]. This is due to the systemic vascular dysregulation present in the course of e.g. hypertension and hypotension, nocturnal hypotension, vasospastic syndromes, blood coagulation disorders, diabetes, hyperlipidemia and hyperthyroidism [44]. Gradual damage to retinal ganglion cells and their apoptosis leads to irreversible atrophic changes in the optic nerve and progressive defects of the visual field [42].

MICRORNA IN RETINAL GANGLION CELLS

Retinal ganglion cells receive visual stimuli from photoreceptors via bipolar and amacrine cells. Axons of retinal ganglion cells form a layer of nerve fibers from which the optic nerve is formed. Damaged cells of this type cannot regenerate, leading to optic neuropathy and loss of vision [45].

Research has shown that miRNAs are involved in regulation of function of retinal ganglion cells. In H_2O_2-induced oxidative stress, miR-100 upregulation and apoptosis of retinal ganglion cells occurred. Contrary, downregulation of miR-100 via lentiviral vectors protected against apoptosis and promoted growth of axons in retinal ganglion cells. In this process, activation of the receptor for the protein secreted by neurons – BDNF (*brain-derived neurotrophic factor*) – was observed [46]. This factor is one of the main neurotrophins, it has a trophic effect on neurons and influences formation of dendrites and axons [47]. In physiological conditions, retinal ganglion cells receive neurotrophins from retinal glial cells (Müller cells) and by retrograde axonal transport from the brain. High IOP disturbs the retrograde transport of neurotrophins in the optic disc, which increases susceptibility of retinal ganglion cells to damage caused by physical or chemical factors [48]. An interaction between miR-100 and the insulin-like growth factor receptor has also been demonstrated. Downregulation of this receptor protects against apoptosis of retinal ganglion cells [46].

Table 1 lists miRNA molecules along with the site of expression and the influence they exert on the pathogenesis of glaucoma.

TABLE 1

The role of microRNA in the pathogenesis of glaucoma.

microRNA	Site of expression	Targets	Action
miR-183	trabecular meshwork	integrin β_1	physiology of trabecular meshwork
miR-29	trabecular meshwork	ECM proteins	ECM homeostasis
miR-144-3p	trabecular meshwork	fibronectin	ECM homeostasis
miR-24	trabecular meshwork	furin	ECM homeostasis
miR-200c	trabecular meshwork	trabecular meshwork cells proteins	contractility of trabecular meshwork cells
miR-143 miR-145	trabecular meshwork	trabecular meshwork cells proteins	contractility of trabecular meshwork cells
miR-17-5p	trabecular meshwork	suppressor protein, Bcl-2, Bcl-xL, Bax	apoptosis of trabecular meshwork cells
miR-100	retina	retinal ganglion cells receptors	apoptosis of trabecular meshwork cells

Bax – B-cell lymphoma-associated X protein; Bcl-2 – B-cell lymphoma-2; Bcl-xL – B-cell lymphoma-extra large; ECM – extracellular matrix.

COMMUNICATION BETWEEN THE ANTERIOR AND POSTERIOR SEGMENTS OF THE EYEBALL

Trabecular meshwork and the lamina cribrosa, through which the optic nerve leaves the eyeball, share neuroectodermal embryogenesis. Presence of neuropeptides secreted by neurons in aqueous humour of patients with glaucoma confirms existence of a molecular connection between the anterior and posterior segments of the eyeball [49]. Neuropeptides act as transmitters of information between neurons and regulate their physiology. They can also modulate gene expression [50]. Differences in protein composition of aqueous humour have also been demonstrated in various eye diseases, both of the anterior and posterior segment, which may suggest existence of a communication pathway between these segments [51]. In patients with glaucoma, proteins are released into aqueous humour from trabecular meshwork cells damaged by oxidative stress. In aqueous humour of patients with POAG, increased activity of nestin, protein kinase A and the actin-binding protein complex has been demonstrated [42]. In another study, in the group of patients with primary closed-angle glaucoma an increased expression of the inflammatory protein – annexin A1, and a decreased expression of the protein associated with cell adhesion – cadherin 4, was demonstrated, compared with those with cataract [52].

Aqueous humour is secreted by the non-pigmented epithelial cells of the ciliary body into the posterior chamber of the eye, then flows through the pupil into the anterior chamber and is drained outside, where it enters systemic circulation. Communication between the anterior and posterior segments of the eye is mediated by the uveoscleral outflow pathway, in which aqueous humour leaves the anterior chamber by diffusion through intercellular spaces between fibers of the ciliary muscle, and then into the suprachoroidal space, scleral canals and conjunctival lymphatic vessels. It has been shown that even large particles can penetrate the suprachoroidal space and the posterior part of the eyeball. One of the studies used radiolabeled albumin, which was detected in the optic nerve region [53]. Perhaps, an unknown mediator exist that transmit signals from the anterior segment of the eye to the retina in the optic disc area.

MICRORNA EXPRESSED IN THE ANTERIOR AND POSTERIOR SEGMENT OF THE EYEBALL

In addition to proteins, trabecular meshwork cells damaged by oxidative stress also release miRNA molecules that act on trabecular meshwork and regulate activation of glial cells, which is an important process in pathogenesis of glaucoma [42]. Among activated glial cells are: astrocytes, Müller cells and microglial cells. Factors triggering their activation involve increased IOP and retinal ischemia. Glial cells respond to stress by changes in distribution and morphology – they reduce the size of the cell body, create thinner branches and increase the number of intercellular connections. Expression of various cytokines or phagocytic activity occurs, resulting in damage to retinal ganglion cells and their apoptosis. Microglia plays a special role, as it lies in the proximity of all elements of retinal ganglion cells: dendrites, synapses, cell bodies and axons. It was proved that in patients with glaucoma, activated microglial cells accumulated in the optic disc area, which is the site of initial axonal damage [54]. Another study showed that in conditions of increased hydrostatic pressure and ischemia, TNF-α (*tumor necrosis factor α*) and nitric oxide are released from glial cells. They exert a cytotoxic effect on retinal ganglion cells, inducing their apoptosis [55].

Among miRNAs released into aqueous humour from damaged trabecular meshwork cells are miR-21, miR-450, miR-107, and miR-149.

It was shown that miR-21, which is upregulated by ROS, reduced apoptosis of trabecular meshwork cells and inhibited microglia activation through extracellular kinase and nuclear factor pathways.

MicroRNA-450 influences the contractile part of trabecular meshwork, activating the myogenic factor that is observed in aqueous humour of glaucoma patients. This factor participates in the control of myoblast differentiation and has a pro-apoptotic effect in them, thus regulating contractility of trabecular meshwork cells. Moreover, miR-450 is an activator of glial cells.

In hypoxic conditions, miR-107 expression increases in trabecular meshwork endothelial cells, preventing their apoptosis. MicroRNA-107 induces apoptosis of glial cells, and also inhibits their activation with the participation of a specific protein – nestin.

MicroRNA-149 controls pathways involved in the metabolism of mitochondria, which are significantly damaged in glaucoma. Its action is mediated by cytokines (TNF-α, interleukin 6) and enzymes (metalloproteinase 9, nitric oxide synthase) [42]. TNF-α, which is elevated in aqueous humour in glaucoma patients, contributes to microglia activation, loss of optic nerve oligodendrocytes and subsequent loss of retinal ganglion cells [56]. Interleukin 6, produced by retina microglial cells in response to increased IOP, protects retinal ganglion cells from apoptosis [57]. Increased activity of metalloproteinase 9 facilitates aqueous humour outflow through changes in ECM proteins, and also induces apoptosis of retinal ganglion cells [58]. Overproduction of nitric oxide by nitric oxide synthase contributes to development of neurodegenerative changes through apoptosis of retinal ganglion cells [59].

Table 2 lists the miRNA molecules along with their site of expression and the influence they exert on pathogenesis of glaucoma.

MicroRNA in gene therapy

Thanks to the advancement in technology, gene therapy is increasingly used in the treatment of genetic diseases. It consists in modifying gene expression or introducing new genes into the organism. Viruses – mainly retroviruses, adenoviruses and lentiviruses – and plasmids, i.e. cellular structures derived from bacteria, are used as vectors of genetic material. Depending on the expected effect, the genetic material used is either DNA or RNA. Two methods of delivering modified vectors to the body are used: *in vivo* and *ex vivo*. In the *in vivo* method, the gene preparation is administered directly into blood vessels or a specific tissue or organ. The *ex vivo* method consists in taking cells from the organism, which are genetically modified in the laboratory, and then multiplied and reintroduced into the patient's body [60].

TABLE 2

The role of microRNA expressed in the anterior and posterior segment of the eyeball in the pathogenesis of glaucoma.

microRNA	Site of expression	Targets	Action
miR-21	trabecular meshwork	–	apoptosis of trabecular meshwork cells
	retina	extracellular kinase, nuclear factor	microglia activation
miR-450	trabecular meshwork	myogenic factor	contractility of trabecular meshwork cells
	retina	–	glial activation
miR-107	trabecular meshwork	–	apoptosis of trabecular meshwork cells
	retina	–	apoptosis of glial cells
	retina	nestin	glial activation
miR-149	retina	TNF-α	microglia activation
	retina	TNF-α	oligodendrocytes
	retina	interleukin 6	apoptosis of retinal ganglion cells
	trabecular meshwork	metalloproteinase 9	ECM homeostasis
	retina	metalloproteinase 9	apoptosis of retinal ganglion cells
	retina	nitric oxide synthase	apoptosis of retinal ganglion cells

ECM – extracellular matrix; TNF-α – tumour necrosis factor α.

CONCLUSION

Research confirms the role of miRNA in the pathogenesis of glaucoma, as well as many other ophthalmic diseases, such as cataract, age-related macular degeneration, diabetic retinopathy, Fuchs dystrophy, retinoblastoma and uveal melanoma [10]. Therefore, miRNA seem to be a potential target of gene therapy. However, since one type of miRNA can regulate expression of many genes, and that multiple miRNA types can act on a single gene, a complicated system arises, in which interference could have immense adverse effects. Therefore, further research is needed to better understand mechanisms of miRNA action and its functions in the body, and to develop an effective and safe gene therapy in the future.

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