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REVIEW

MicroRNAs in glaucoma and neurodegenerative diseases

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MicroRNAs (miRNAs) constitute a class of short, non-coding RNAs, which have important role in post-transcriptional regulation of genes expression by base-pairing with their target messenger RNA (mRNA). In recent years, miRNAs biogenesis, gene silencing mechanism and implication in various diseases have been thoroughly investigated. Many scientific findings indicate the altered expression of specific miRNA in the brains of patients affected by neurodegenerative diseases (NDs) such as Alzheimer's disease, Parkinson's disease and Huntington disease. The progressive optic nerve neuropathy associated with changed miRNA profile was also observed during glaucoma development. This suggests that the miRNAs may have a crucial role in these disorders, contributing to the neuronal cell death. A better understanding of molecular mechanism of these disorders will open a new potential way of ND treatment. In this review, the miRNAs role in particular neurodegenerative disorders and their possible application in medicine was discussed.
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INTRODUCTION

MicroRNAs (miRNAs or miRs) are endogenous, non-coding RNAs. They have a single-stranded structure and ~22 nucleotides (nt) in length.[1–3] The main function of the miRNAs is the post-transcriptional regulation of protein-coding gene expression by binding to the 3′untranslated region (UTR), the coding sequence or the 5'UTR of targeted messenger RNA (mRNA), which leads to the inhibition of translation process or mRNA degradation.[1,4]

Presumably, the expression of more than one-third of human genes is regulated by miRNAs.[5] The known functions of miRNAs include the control of cell proliferation, differentiation, apoptosis and metastasis.[6–8] They also act as the regulatory factors of diverse biological pathways including developmental timing control[2] and hematopoietic lineage differentiation in mammals.[9] Recent studies have shown their implication in pathogenesis of neurodegenerative diseases (NDs) and carcinogenesis.[4,10]

Lin-4, the first identified miRNA, was discovered in *Caenorhabditis elegans* in 1993 by Ambros and Ruvkun. They found that its activity was crucial for the transition from the first larval stage to the second stage. Seven years after this finding, another miRNA (*lin-7*) was reported as a controlling factor of the L4-to-adult transition in nematode.[4] Since that, many miRNAs have been discovered in viruses, plants, animals and humans.[3,11] It was estimated that miRNAs constitute nearly 1% of all predicted genes.[10,12] Both miRNA sequence and the miRNA processing machinery demonstrated high evolutionary conservation. That suggests that miRNAs have critical regulatory function in all living organisms.[11]

The miRNA processing machinery

In the nucleus, miRNA genes are mainly transcribed by RNA polymerase II into primary miRNA (pri-miRNA), although the activity of RNA polymerase III was also reported.[13] The pri-miRNA transcripts form the stem-loop structures, ended with hairpin shape (Figure 1). They are often several hundred nucleotides long with a fragmentary complementary sequence in the stem region. The pri-miRNA is cleaved by the nuclear microprocessor complex composed of the highly conserved RNase III Drosha and the DGCR8 (DiGeorge critical region 8) protein.[2,14] The Drosha enzyme cleaves the 5′ and 3′ arms of the pri-miRNA, while the DGCR8 role is to find the precise cleavage sites.[13]

The precursor-miRNA (pre-miRNA) obtained in this way is usually characterized by ~70 nt length.[11] After nuclear processing, the pre-miRNA is recognized by export receptor Exportin5 (XPO5) and actively transported by Ran-GTP complex from nucleus through the nuclear pore complex to the cytoplasm.[2,13] Next, the Dicer endoribonuclease causes the removal of pre-miRNA loop and its transformation into 19–24 nt mature double-stranded miRNA, which is able to regulate gene expression post-transcriptionally.[11,14]

Post-transcriptional gene silencing by miRNA

The miRNA action proceeds through a ribonucleoprotein structure, called miRNA-induced silencing complex (miRISC) (Figure 1).[11] It comprises a single-stranded miRNA and proteins forming the RNA-induced silencing complex (RISC) loading complex, built of Dicer enzyme, TRBP (Tar RNA binding protein) and Argonaute-2 (Ago2) protein.[13]

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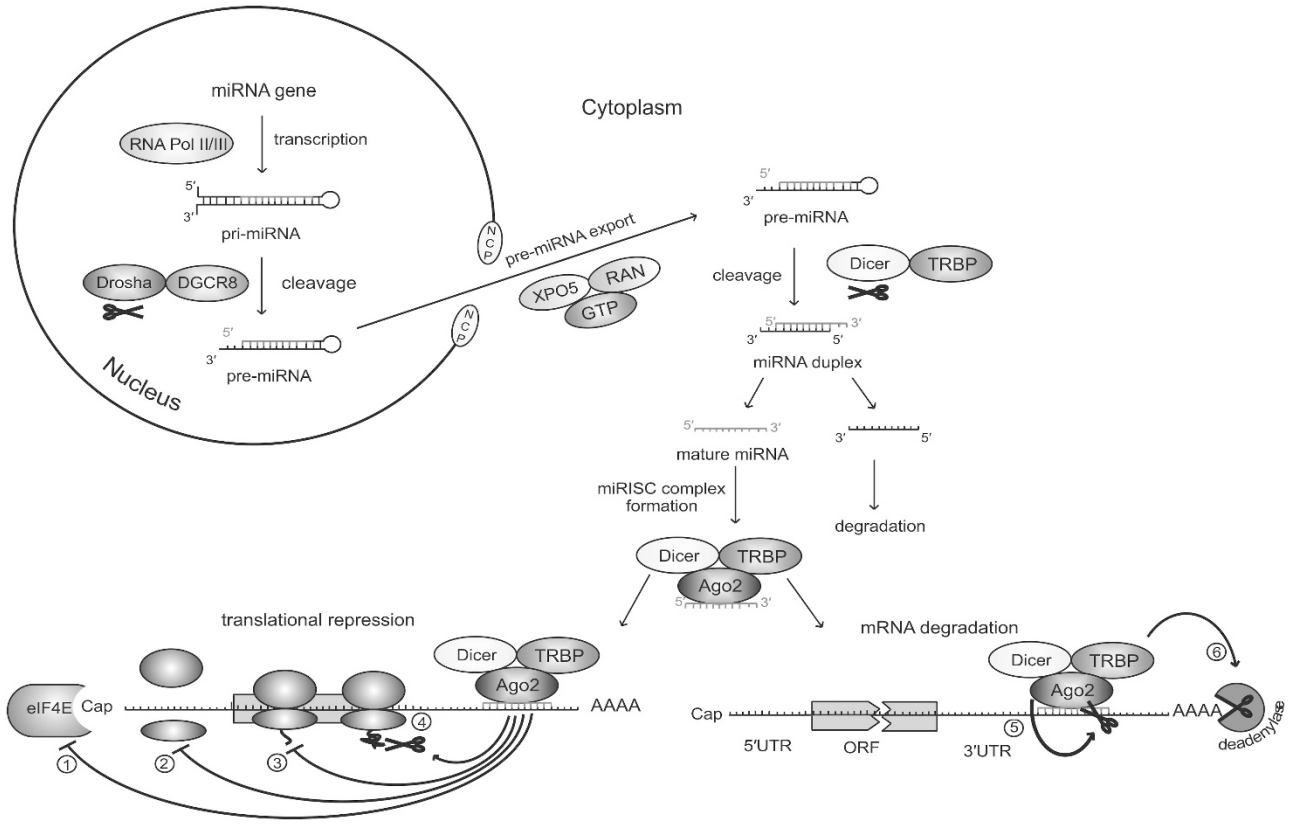

Figure 1 miRNA biogenesis and the silencing mechanism. miRNA processing begins in the nucleus, where miRNA gene is transcribed by RNA polymerase either II or III. Created pri-miRNA is cleaved by Drosha–DGCR8 complex into pre-miRNA and then transported into the cytosol by XPO5, Ran-GTP proteins. Dicer, with the support of the dsRNA-binding domain TRBP, cleaves the loop, leaving miRNA duplex. The functional miRNA strand is loaded into the RISC, where it recognizes the mRNA 3′UTR target. The miRISC performs translational repression via (1) competition with the eIF4E initiation factor, (2) inhibition of ribosomal subunits association, (3) premature termination of polypeptide synthesis or (4) degradation of newly-synthesized polypeptide. mRNA degradation induced by miRISC could be performed due to (5) mRNA cleavage or (6) deadenylation. The scheme is based on Winter *et al.*,[13] Araldi *et al.*[14] and Wu *et al.*[16] RNA Pol, RNA polymerase; pri-miRNA, primary RNA; pre-miRNA, precursor RNA; XPO5, Exportin5; DGCR8, DiGeorge critical region 8; TRBP, Tar RNA binding protein; Ago2, Argonaute-2, RISC, RNA-induced silencing complex. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Dicer belongs to the RNase III family and it cleaves double-stranded RNA (dsRNA) into small fragments.[15] TRBP is a dsRNA-binding domain, which facilitates Dicer activity by its stabilization. TRBP is not indispensable for the post-transcriptional gene silencing; however, its scarcity reduces the efficiency of this process. Furthermore, TRBP takes part in Ago2 recruitment, which is the RISC loading complex core component.[13]

One strand of the mature miRNA is incorporated into RISC and it indicates precisely the cutting target. Usually, it is the guide strand, characterized by lower thermodynamic stability. The passenger strand could also potentially generate functional miRNA, however in most cases it is degraded.[2,14]

As soon as the miRISC finds target 3′UTR, it can repress translation or cleavage the mRNA. The most essential sequence in the miRNA is the miRNA seed region. It has an important role in pairing with the target mRNA. Usually, it is localized at position 2–8 nucleotides in the 5′ end of miRNA and it exhibits almost perfect complementation to 3′UTR sequence.[13]

The crucial element in translational repression is Ago2, which can bind to the mRNA Cap, competing with initiation factor eIF4E. Recently, the other mechanisms of translational repression by miRNA have been also proposed, including blocking ribosomal subunits association, premature termination of polypeptide synthesis and degradation of newly produced chain.[16]

miRNA can also trigger the mRNA decay via two different mechanisms. In the first one, mRNA is cleaved within the base-paired region due to endonucleolytic activity of Ago2. Alternatively, miRNA interacts with mRNA through removal of the 3′poly (A) tail. In the consequence of deadenylation, mRNA is exposed to exonucleolytic digestion from the 5′ end.[14,16]

MIRNAS IN NEURODEGENERATION

Neurodegeneration refers to the progressive loss of neurons structure and function, resulting in their death.[17] It also refers to the retinal ganglion cell (RGC) death; therefore, glaucoma is often considered as a ND.[18] NDs become one of the most serious health problems nowadays, especially in the light of population aging phenomenon. Despite many years of research, there is no effective treatment and NDs are still considered as incurable and irreversible, with devastating consequences for patients and their environment.

The molecular background of these disorders is complex and many events are involved in their development, that is, oxidative stress, axonal transport deficits, protein oligomerization and aggregation, calcium deregulation, mitochondrial dysfunction, neuron–glial

interactions, neuroinflammation, DNA damage and aberrant RNA processing.[19,20]

Recent data demonstrated the significant alterations of miRNAs level in NDs pathogenesis and their contribution to abnormal neuronal physiology.[17,19] In different types of cells, in the Dicer absence, the neurodegradation through cell-autonomous and non-cell-autonomous mechanisms was observed. Dysregulated miRNAs expression occurred frequently in patients suffering from these types of illnesses, which suggested that the miRNAs were another considerable factor facilitating the development of NDs.[21,22]

In this review, we discussed the miRNAs implications in neuropathy in the pathogenesis of the most common neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and glaucoma.

miRNAs in AD

AD is prevalent and the most explored form of degenerative disease of the central nervous system.[22] The hippocampus and subcortical structures are the most affected areas of the brain, which results in AD symptoms such as memory impairments, as well as progressive language and cognition difficulties.[19,22] The main neuropathological hallmark of AD is the deposition of intracellular neurofibrillary tangles, containing hyperphosphorylated Tau protein and extracellular accumulation of neurotoxic plaques formed by β-amyloid (Aβ) peptides. Latter are obtained from proteolytic activity of amyloid precursor protein (APP) by both β-site APP-cleaving enzyme 1 (BACE1) and the intermembranous γ-secretase complex.[23,24] The formation of neurofibrillary tangles is largely associated with Aβ toxicity. The soluble form of Aβ is involved in cleavage and phosphorylation of Tau through activation of enzymes crucial in these processes, including kinases GSKβ and cdk5.[25] According to the current state of knowledge, the proteolysis of APP seems to be the important event in AD pathogenesis; however, the physiological role of APP remains uncertain. It is presumed that it could be responsible for cell growth modulation, mortality, neurite outgrowth and survival.[24] The literature data indicate that miRNAs probably regulate the expression of genes involved in Aβ formation.[26,27]

APP as well as BACE1 mRNAs contain miRNA target sites in their 3′UTRs. It enables direct regulation of APP synthesis by the following miRNAs: miR-106a, miR-520c, miR-16, miR-101, miR-147, miR-655, miR-323-3p, miR-644 and miR-153.[19] However, the identification of most of mentioned miRNAs has been performed *in vitro* (Table 1).

It has been demonstrated that BACE1 expression is modulated by some specific miRNAs such as miR-29a, miR-29b-1, miR-9, miR-29c, miR-298, miR-328, miR-195, miR-124 and miR-107.[26,28–30] Performed investigation showed that the low expression of miR-29a/b-1 was correlated with upregulated BACE1 protein level in brains of sporadic AD patients. It suggested that the loss of miR-29a and miR-29b-1 presumably contributed to sporadic AD development.[26] Zong *et al.*[28] demonstrated that another member of miR-29 family, miR-29c, was also implicated in BACE1 expression regulation but it exhibited the opposite effect. The overexpression of miR-29c was associated with downregulated BACE1 expression in mice, which made it an interesting novel target in AD therapy. Similar results were obtained by Fang *et al.*,[30] showing that miR-124 inhibited the BACE1 protein expression in PC12 cell lines and primary cultured hippocampal neurons. A decreased neuronal miR-107 expression with simultaneous increase of BACE1 level have been detected in patients with AD in both early and advanced stage.[27,31] Furthermore, this miRNA also controlled expression level of cofilin—another protein associated with AD pathogenesis.[32] Published data indicated that some members of miR-16 family: miR-16, miR-15, miR-195, miR-497 could regulate endogenous extracellular signal-regulated kinases (ERK1) and influence Tau phosphorylation. The investigations have been performed *in vitro* in neuronal cells and revealed that also miR-15 directly targets ERK1.

Tau kinase is classified as ERK1. The alterations in miR-15 expression potentially could be associated with abnormal Tau phosphorylation occurring in AD.[33] Moreover, splicing regulation of the endogenous Tau exon 10 is also modulated by miR-132.[33] Dickson *et al.*[34] found miRNA-binding sites in the Tau 3′-UTR and showed that miR-34a targets this region decreasing Tau expression. This action exhibits potential therapeutic applications in AD treatment. The examples described above confirm that miRNA deregulation is the crucial mechanism contributing to AD initiation and progression.

miRNAs in PD

PD is the second most common neurodegenerative disorder. Available estimates show that it affects ~1% people after 60 years of age.[35] The major characteristic features are loss of dopaminergic neurons (DNs) in the substantia nigra and the deposition of Lewy bodies of α-synuclein (SNCA) in this area. These intracellular inclusions negatively affect the neuron survival, leading to clinical symptoms such as progressive rigidity, bradykinesia and resting tremor.[36] The knowledge in the field of genetics of PD is still strictly limited. The available data highlight the mutations in *SNCA, PARKIN, UCHL-1, PINK1, DJ-1* and *LRKK2* genes as an important factor in familiar form of this illness; however, these cases are rare.[22]

Scientific findings from recent years indicate miRNAs implication in DNs differentiation and their contribution to PD development.[37–39] It has been proven that the deletion of *Dicer* significantly decreased the differentiation of embryonic stem cells into DNs, while the transfection of miR-133b obtained from the embryonic mouse midbrain enhanced this process.[37] Moreover, the association between DNs survival and miRNAs action has been demonstrated in both cultured cells and *in vivo*. The deletion of *Dicer* in mouse midbrain DNs caused DNs death and the appearance of symptoms characteristic for PD. The comparison of miR-133b expression profiles of normal midbrain with these obtained from PD individuals showed that the decreased expression in brains affected by PD.

Performed investigations established the miR-133b function as a negative regulation of DNs differentiation by modulating the expression of Pitx3 transcription factor (TF).[37,40] The proteins encoded by genes correlated with PD, α-synuclein and leucine-rich repeat kinase 2 (LRRK2), are regulated by specific miRNA. The inhibition of SNCA could be performed by miR-7 and miR-153 due to direct binding to the 3′UTR of SNCA mRNA.[41,42] Mentioned miRNAs cooperate with each other to control amount of produced synuclein in the brain.[43] It has been demonstrated that mutations in LRRK2 are responsible for DNs degeneration and PD development. It has been observed that LRRK2 is engaged in the negative regulation of *Ago1* and *Ago2*, causing a disruption in the synthesis of E2F1, DP and the miRNA.

Gehrke *et al.*[44] stated the miR-184 and let-7 role in translational repression of *Drosophila* E2F1 and DP. They also observed that increased levels of this two miRNAs reduced negative LRRK2 effects. *In silico* analysis determined a predicted binding site for miR-205 in the 3′UTR region of *LRKK2* gene. Later *in vitro* studies performed in the commercial cell lines and primary neuron cultures established a direct inhibition of *LRKK2* via miR-205. Based on these findings, it may be concluded that downregulation of miR-205 presumably had impact on increased LRRK2 protein level in PD patients.[45]

Table 1 List of selected miRNAs involved in neurodegeneration with their molecular targets

miRNA	*ND*	*Molecular target*	*Type of experiment*	*Mechanism*	*Reference*
-106a, -520c	AD	APP	*In vitro* reporter assays	Regulation of APP levels	94
-16	AD	APP	Profiling in mouse model	Regulation of APP levels	95
-101	AD	APP	*In vitro* reporter assays	Regulation of APP levels	96
-147, -655, -323-3p, -644, -153	AD	APP	*In vitro* reporter assays	Regulation of APP levels	97
-29a, -29b-1, -9	AD	BACE1	*In vitro* reporter assay, profiling in patients	Regulation of BACE1 levels	26
-29c	AD	BACE1	Profiling in mouse model	Regulation of BACE1 levels	28
-298/-328/ -195	AD	BACE1	Profiling in patients	Regulation of BACE1 levels	98
-124	AD	BACE1	*In vitro* reporter assay	Regulation of BACE1 levels	30
-107	AD	BACE1	Profiling in patients, *in vitro* reporter assay, *in situ* hybridization in patients	Regulation of BACE1 levels Repression of cofilin translation	32,39
-16, -15, -195, -497	AD	ERK1 and Tau	Profiling in mouse model	Regulation of ERK1 and Tau levels	33
-132	AD	PTBP2	Profiling in mouse model	Regulation of neuronal splicing of Tau Exon 10	33
-34a	AD	Tau	*In vitro* reporter assay	Regulation of Tau levels	34
-133b	PD	Pitx3	Profiling in patients	Regulation of the maturation and function of midbrain dopaminergic neurons	37
-7	PD	DP	*In vitro* reporter assay	Regulation of α-synuclein levels	41,42
-153	PD	E2F1	*In vitro* reporter assay	Regulation of α-synuclein levels	41
-184	PD	LRRK2	Profiling in Drosophila	Regulation of DP messenger RNAs synthesis	44
let-7	PD	LRRK2	Profiling in Drosophila	Regulation of Drosophila e2f1 synthesis	44
-205	PD	LRRK2	Profiling in patients	Regulation of LRRK2 protein levels	45
-433	PD	SNP rs12720208 in the 3′ UTR	Profiling in patients	Regulation of FGF20 expression and α-synuclein levels	39
-9/-9*	HD	REST/CoREST	Profiling in patients	Double negative feedback loop between the REST silencing complex and the miRNAs	55
-9-1, 9-3, 29a, 29b-1, 124a-1, 124a-2, 124a-3, 132, 135b, 139, 203, 204, 212, 330, 346	HD	REST	Profiling in mouse model, profiling in patients	Dysregulation of transcriptional and post-transcriptional mechanisms	52
-29b	Glaucoma	BMP1, ADAM12, NKIRAS2	*In vitro* reporter assay	Negative regulation of the expression of ECM components in TM cells	67
-200c	Glaucoma	ZEB1, ZEB2, FHOD1, LPAR1/ EDG2, ETAR, RHOA	*In vitro* reporter assay, transfection assay in rat model	Post-transcriptional inhibition of target genes, IOP regulation	69
-204	Glaucoma	FOXC1	*In vitro* reporter assay	Reduction of *FOXC1* expression, leading to *CLOCK, PLEKSHG5, ITGβ1, MEIS2* decreased expression	70
-24	Glaucoma	FURIN	*In vitro* reporter assay	Downregulation of FURIN, leading to decreased activity of TGFβ1	71

Abbreviations: APP, amyloid precursor protein; BACE1, β-site APP-cleaving enzyme 1; ECM,extracellular matrix; FOXC1, Forkhead box C1; IOP, intraocular pressure; LRRK2, leucine-rich repeat kinase2; miRNA, MicroRNA; ND, neurodegenerative disease; SNP, single-nucleotide polymorphism; TGFβ1, transforming growth factor beta 1; TM, trabecular meshwork; UTR, untranslated region.

The interactions between miRNAs and their targets could be affected by the single-nucleotide polymorphism occurrence. The polymorphic variants in the *fibroblast growth factor 20* (*FGF20*) are potentially connected with PD pathogenesis. The main role of *FGF20* is supporting DNs survival. The single-nucleotide polymorphism rs127202208, located within the *FGF20* 3′UTR, which is a predicted binding site for miR-443, was detected predominantly in PD patients.[39] Wang *et al.*[39] have shown that the risk allele of rs12720208 disturbs miR-443 binding, resulting in enhanced translation of FGF20. In consequence, the elevated α-synuclein expression occurred, which was associated with the PD development.

miRNAs in HD

HD belongs to the group of polyglutamine (polyQ) diseases. HD affects about 3 in 100 000 people and early symptoms occur between the age of 35 and 50 years.[46] The palliative care is the main way of HD treatment, due to lack of effective methods of causative therapy.[19] HD is induced by an unstable CAG repeats accumulation in the first exon, on chromosome 4 of a gene encoding the huntingtin protein (Htt).[47] It is characterized by the progressive loss of cortical and striatal neurons.[48,49] The toxicity of mutant Htt protein causes brain neurons death, which results in the following symptoms: motor impairments, cognitive and behavioral defects. The physiological role of Htt was not clearly indicated.[48,50] Interestingly, the mutant Htt can interact with Ago1 and Ago2, leading to the inhibition of the processing bodies (P bodies) formation, which suggests that Htt could have role in gene silencing mechanism mediated by miRNA.[51]

Published data showed significant number of evidence indicating a crucial role of miRNAs in HD.[52–55] It has been demonstrated that Htt interacts with repressor element 1 silencing transcription factor (REST). In healthy people, Htt isolates REST in the cytoplasm of neurons, preventing the repressor from binding to DNA. In HD, REST is transferred to the nucleus of neuron and represses many of its target genes, including *BDNF*, which is responsible for neuron survival.[56]

Scientific findings demonstrated that REST together with its cofactor (coREST) has target sites for miR-9 and miR-9*. Moreover, miR-9 and miR-9* are downregulated in HD-affected subjects.[51,53] Johnson *et al.*[52] identified numerous neuron-specific REST-target miRNAs in the human genome; miR-9-1, 9-3, 29a, 29b-1, 124a-1, 124a-2, 124a-3, 132, 135b, 139, 203, 204, 212, 330 and 346. Four of them (miR-29a, miR-124a, miR-132 and miR-330) exhibited decreased expression level in the cortex of HD mice. The expression profile of miR-29a, miR-124a, miR-132 and miR-135b in human parietal cortical tissues has been analyzed. This investigation revealed miR-132 downregulation in HD patients, while the expression of miR-29a and miR-330 was increased. No differences between HD and control individuals for miR-124a have been detected.[52]

Performed qRT-PCR miRNA experiment showed deregulation of miRNAs including miR-486, miR-196a, miR-17-3p, miR-22, miR--485-5p, miR-500 and miR-222 in the cortex of patients with early stage of HD.[55] Overexpression of specific miRNA in the frontal cortex and striatum in HD patients was also found for miR-100, miR-151-3p, miR-16, miR-219-2-3p, miR-27b, miR-451 and miR-92a during HD development, whereas decreased level was detected for miR-128, miR-139-3p, miR-222, miR-382, miR-433 and miR-483-3p.[54]

Recent studies provided more evidence to support the theory that miRNAs are engaged in HD. Bañez-Coronel *et al.*[57] discovered the mutant Htt CAG repeats interfere with neuronal viability at the RNA level. Small RNAs from Htt mutant cells and from the brain tissue from HD patients increased neurodegeneration, in an Ago2-dependent mechanism.

Recent investigation indicated a significant number of miRNAs, which expression was altered in HD individuals comparing with the controls. Fifty-four selected miRNAs were investigated and the expression level of 30 miRNAs was increased in HD patients. Decreased expression was detected for 24 miRNAs.[58] Deregulation of 33 miRNAs was connected with HD occurrence and associated with altered level of TFs in the HD brain, including TP53, REST, E2F1 and GATA4.[58] Multiple miRNAs are implicated in HD pathogenesis and development, thus this topic demands further investigation. All the data discussed in above three chapters are summarized in Table 1.

miRNAs in glaucoma

Glaucoma is an ocular disorder, characterized by progressive and irreversible optic nerve degeneration. The pathogenesis of glaucoma is not fully elucidated yet; however, in most cases it is associated with elevated level of intraocular pressure (IOP), which leads to degeneration of the optic nerve via RGC death. In a consequence, the glaucoma patients struggle with visual field loss or if the disease is advanced with blindness.[59,60]

The most common type of this illness is primary open-angle glaucoma (POAG), in which optic neuropathy occurs without clearly identified secondary cause.[59,61] It is presumed that crucial role in PAOG pathogenesis has increased IOP caused by the impairment in aqueous humor (AH) outflow through trabecular meshwork (TM).[62] TM is a structure responsible for draining AH from the eye and producing extracellular matrix (ECM). Any disruption in the balance between ECM synthesis and breakdown may change aqueous outflow and contribute to glaucoma development.[63,64]

miRNAs role in modulating cellular function of TM in normal and pathological conditions has been revealed. It is supposed that they may be implicated in ECM turnover, also during glaucoma pathogenesis. It has been shown that there is a communication between TM and glial cells, which may be additional mechanism underlying the neurodegeneration in POAG.[65] However, the current knowledge in this field is limited.

Scientific findings suggest that miRNA-29 family, implicated in AD and HD pathogenesis, may also has a role in the regulation of ECM homeostasis in TM. Villarreal *et al.*[63] indicated the miRNA-29 family acts as a repressor of diverse ECM proteins in normal condition and under stimulation of the transforming growth factor beta 2 (TGFβ2). Luna *et al.* demonstrated that miR-29b negatively regulated the expression of genes involved in ECM metabolism in TM cells, including multiple collagens, fibrillins and elastin.[66,67] Downregulation of miR-29b presumably contributed to elevated expression of several ECM genes under chronic oxidative stress conditions (Table 1). This may lead to increased deposition of ECM in the TM and in a consequence disturbed AH outflow characteristic of glaucoma.[67] Moreover, the published data revealed that miR-29 family could be implicated in modulation of the transforming growth factors beta (TGFβs). The alternation of TGFβ levels was often observed in glaucoma. Performed study showed that TGFβ1 did not affect miR-29b and miR-29c, and upregulated miR-29a, while TGFβ2 significantly decreased the expression of all of them. This down-regulation with the inhibitory effect of miR-29b on the expression of TGFβ1 suggested that changed expression of miR-29 family might cause upregulation of ECM genes induced by TGFβ2 in TM.[68]

Recent findings presented evidence that miR-200c could regulate trabecular contraction and IOP *in vivo*. Cellular contraction is responsible for decreasing TM permeability and AH from the eye by

minimizing the intercellular spaces. The mechanism of miR-200c action is probably based on direct post-transcriptional inhibition of genes associated with TM cells contraction regulation including following targets: Zinc finger E-box binding homeobox 1 and 2 (ZEB1 and ZEB2), formin homology 2 domain containing 1 (FHOD1), lysophosphatidic acid receptor 1 (LPAR1/EDG2), endothelin A receptor (ETAR) and RhoA kinase (RHOA).[69]

Paylakhi *et al.*[70] performed microarrays expression analysis to identify genes in TM cell primary cultures, which expressions were affected by Forkhead box C1 (FOXC1)—a TF believed to be implicated in glaucoma development. Further studies demonstrated that miR-204 caused reduced expression of *FOXC1* and the *FOXC1* target genes *CLOCK, PLEKSHG5, ITGβ1* and *MEIS2*. This suggested that the gene expression in the TM was regulated by a complex network, in which various TFs such as FOXC1 and miRNAs including miR-204 might be involved.[70]

Different factors, such as cyclic mechanical stress, led to the alterations in the TM cells, for instance the upregulation of TGFβ1. The phenomenon may be potentially one of the reasons contributing to the glaucoma. Changes in the miRNA profile evaluation during cyclic mechanical stress revealed that the overexpression of miRNA-24 caused reduced activity of TGFβ1 through direct miR-24 targeting of the subtilisin-like protein convertase FURIN. It supports the theory that the miRNAs may modulate cellular functions in the TM.[71] A group of proteins involved in ECM remodeling are matrix metalloproteinases (MMPs). Matrix metalloproteinase 9 (MMP-9) action is linked to apoptosis in RGC. Abnormal activity of MMP-9 is caused by change in its expression and is associated with glaucoma. Performed studies demonstrated that 3′UTR of MMP-9 has targets for miRNA, which regulates this enzyme.[72]

A significant number of studies have been performed to investigate the function and expression profile of specific miRNAs in the retina.[73–75] Recent data showed the alternations in expression level of miRNA-100 in RGCs. Kong *et al.*[76] indicated that the down-regulation of miR-100 had a protective effect on RGC-5 in oxidative stress conditions, preventing them from apoptosis. The elevated expression of miR-100 promoted the neuronal growth of RGC.[76] The data revealed functional role of miR-100 in retina and indirectly suggested that it could be associated with glaucoma pathogenesis, in which RGCs were preferentially damaged. However, the investigation has not been performed in glaucomatous cell lines. It has been shown that the knockout of miR-183/96/182 cluster in mice caused retinal degeneration.[74] These reports implied that miRNAs may have an important role in glaucoma, although retinal tissue from affected individuals have been not studied.

Jayaram *et al.*[77] evaluated the expression profile of miRNAs isolated from retinae of rat eyes with advanced nerve damage induced by elevated IOP. Eight miRNAs were significantly downregulated in glaucomatous retinae compared with controls (miR-181c, miR-497, miR-204, let-7a, miR-29b, miR-16, miR106b and miR-25). On the other hand, miR-27a was significantly upregulated. Observed miRNAs level alterations caused enrichment of targets associated with ECM/cell proliferation, immune system and regulation of apoptosis.

Gao *et al.*[78] investigated the effect of optineurin *OPTN* E50K mutation on miRNAs expression profile in transgenic mice. Mutations in the *OPTN* have been associated with POAG and normal tension glaucoma. Previous studies have shown that E50K OPTN participates in neurodegeneration by induction of apoptosis of RGCs in transgenic mice models and progressive retinal degeneration exclusively in the peripheral region of the retinas.[79] The results obtained by Gao *et al.* suggest that the miRNAs miR-141, miR-200a, miR-200b, miR-200c and miR-429, which all belong to the miR-8 family, may be critical regulators of POAG induced by the *OPTN* (E50K) mutation.

Izotti *et al.*[65] trials reveal, as established both *in vitro* and in glaucomatous AH, that TM cells damaged by oxidative stress release extracellular miRNAs inducing glial cell activation, an established pathogenic mechanism in NDs. Released miRNAs include miR-21 (affecting apoptosis), miR-450 (cell aging, maintenance of contractile tone), miR-107 (Nestin expression, apoptosis) and miR-149 (endothelia and ECM homeostasis).

Tanaka *et al.*[80] performed an analysis of extracellular miRNA profiles in the glaucomatous AH, which showed that 11 miRNAs were significantly upregulated and 18 downregulated ($P<0.05$ for both).

The knowledge in the field of miRNA role in glaucoma is still strictly limited. The literature data show correlation between changed expression of genes essential in glaucoma pathogenesis and different levels of miRNA in eye tissues.[81–83] Altogether, they suggest that the neurodegeneration is linked with altered miRNAs expression; however, the molecular mechanism of miRNA action is mostly unknown.

THE THERAPEUTIC MIRNA APPLICATION IN NDS

Nowadays, the effective therapy for ND is still not known. The neurodegeneration is irreversible and the number of affected people is still increasing.[84–86] Regarding these facts, miRNAs remain a promising tool in the NDs and glaucoma therapy. This single strategy, once developed, could be possibly applied in the treatment of all discussed NDs.

Several approaches are considered to be used as an miRNA-based therapeutics. First one is miRNA mimics application in downregulation of certain target proteins. These RNA molecules resemble miRNA precursors. If the gene does not have endogenous miRNA, naturally occurring in the organism, then there is a possibility of designing artificial miRNAs targeting them. However, this strategy is connected with potential risk of the off-target effects and the probability of designed miRNA interference with those existing in cells, in normal conditions.[87,88]

A second approach to miRNA downregulation is a usage of anti-miRNA molecules to enhance cellular survival pathways and, in a consequence, limitation of cell apoptosis. The specificity of the anti-miRNA molecules has been investigated and increased by locked nucleic acid-modified oligonucleotides. This manipulation inhibits an endogenous miRNA and becomes a promising therapy for ND patients.[87,89]

Another viewpoint on the NDs including glaucoma treatment is the potential possibility of targeting the miRNA processing machinery, hence it is so important to identify the changes in miRNA biosynthesis during neuronal cell death. The knockout of the *Dicer1* in Drosophila neurons induced the process of apoptosis and the deregulation of other compounds of miRNA processing pathways.[90] In the miRNA processing machinery, several pathways are engaged and the biggest obstacle remains design the specific targets useful in NDs therapy.

Since Andrew Fire and Craig Mello were awarded with the Nobel Prize in Medicine for the discovery of dsRNA role in RNA interference (RNAi), the interests in RNAi therapeutics rapidly increased. However, most of the research programs based on RNAi therapeutic technologies performed by companies such as Merck, Roche and Novartis were halted by the end of 2014. The creation of a small-molecule with specific target site is challenging and expensive investment. Usually, it takes at least 5 years to establish a drug candidate for clinical trials and only about 10% of tested molecules are

able to enter the market. In practice, more drugs lose their patents then are approved. All together in the connection with delivery problems of miRNAs and sales prediction made the most of the investors to decide to shut down RNAi programs, without consideration of patients care.[91]

However, the number of scientific reports indicating miRNAs implication in human diseases and their potential application in medical treatment is systematically increasing. The knowledge about molecular mechanism of their action in neurodegeneration and different types of cancer is better. Also, the problems with miRNA delivery seem to be overcoming, in view of rapid nanotechnology development and creation of novel, efficient nanovehicles for siRNA and miRNA delivery.[92] Different miRNA delivery strategies have been developed and tested *in vitro* or *in vivo* including the covalent coupling of RNAs to cholesterol, lipids, peptides, antibodies in order to increase RNA protection, chemical nucleotide modifications in the RNA structure, for instance the inclusion of locked nucleic acids, unlocked nucleic acids, 2′F-, 2′-OMe- or sugar-phosphate backbone-modified nucleotides increasing the RNAs stability. Many nanoparticles have been explored for their utility in RNA delivery such as inorganic nanoparticles, various liposomes and polymers, where the cationic polymers and lipids show highest gene delivering capacity. It seems that polyethylenimines represent a promising tool for the delivery of small RNA molecules; however, they are still subject of study over structure optimization as well as application.[92,93]

The application of miRNAs in therapy of tumors and neurodegradation occurring in NDs and glaucoma seems to be underestimated, but it hides a big potential and there is a strong hope that pharmaceutical companies will again focus their research on this topic.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Schanen, B. C. & Li, X. Transcriptional regulation of mammalian miRNA genes. *Genomics* **97**, 1–6 (2011).
2 Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281–297 (2004).
3 Li, L., Xu, J., Yang, D., Tan, X. & Wang, H. Computational approaches for microRNA studies: a review. *Mamm. Genome* **21**, 1–12 (2010).
4 Almeida, M. I., Reis, R. M. & Calin, G. A. MicroRNA history: discovery, recent applications, and next frontiers. *Mutat. Res.* **717**, 1–8 (2011).
5 Liu, Z., Sall, A. & Yang, D. MicroRNA: an emerging therapeutic target and intervention tool. *Int. J. Mol. Sci.* **9**, 978–999 (2008).
6 Xu, P., Vernooy, S. Y., Guo, M. & Hay, B. A. The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. *Curr. Biol.* **13**, 790–795 (2003).
7 Cho, W. C. S. OncomiRs: the discovery and progress of microRNAs in cancers. *Mol. Cancer* **6**, 60 (2007).
8 Brennecke, J., Hipfner, D. R., Stark, A., Russell, R. B. & Cohen, S. M. bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. *Cell* **113**, 25–36 (2003).
9 Chen, C.-Z., Li, L., Lodish, H. F. & Bartel, D. P. MicroRNAs modulate hematopoietic lineage differentiation. *Science* **303**, 83–86 (2004).
10 Cai, Y., Yu, X., Hu, S. & Yu, J. A brief review on the mechanisms of miRNA regulation. *Genomics Proteomics Bioinformatics* **7**, 147–154 (2009).
11 Etheridge, A., Lee, I., Hood, L., Galas, D. & Wang, K. Extracellular microRNA: a new source of biomarkers. *Mutat. Res.* **717**, 85–90 (2011).
12 Lewis, B. P., Shih, I., Jones-Rhoades, M. W., Bartel, D. P. & Burge, C. B. Prediction of mammalian MicroRNA targets. *Cell* **115**, 787–798 (2003).
13 Winter, J., Jung, S., Keller, S., Gregory, R. I. & Diederichs, S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. Cell Biol.* **11**, 228–234 (2009).
14 Araldi, E. & Schipani, E. MicroRNA-140 and the silencing of osteoarthritis. *Genes Dev.* **24**, 1075–1080 (2010).
15 MacRae, I. J., Zhou, K., Li, F., Repic, A., Brooks, A. N., Cande, W. Z. *et al.* Structural basis for double-stranded RNA processing by Dicer. *Science* **311**, 195–198 (2006).
16 Wu, L. & Belasco, J. G. Let me count the ways: mechanisms of gene regulation by miRNAs and siRNAs. *Mol. Cell* **29**, 1–7 (2008).
17 Nelson, P. T., Wang, W.-X. & Rajeev, B. W. MicroRNAs (miRNAs) in neurodegenerative diseases. *Brain Pathol.* **18**, 130–138 (2008).
18 Gupta, N. & Yücel, Y. H. Glaucoma as a neurodegenerative disease. *Curr. Opin. Ophthalmol.* **18**, 110–114 (2007).
19 Maciotta, S., Meregalli, M. & Torrente, Y. The involvement of microRNAs in neurodegenerative diseases. *Front. Cell. Neurosci.* **7**, 265 (2013).
20 Majsterek, I., Slupianek, A., Hoser, G., Skórski, T. & Blasiak, J. ABL-fusion oncoproteins activate multi-pathway of DNA repair: role in drug resistance? *Biochimie* **86**, 53–65 (2004).
21 Gascon, E. & Gao, F.-B. Cause or effect: misregulation of microRNA pathways in neurodegeneration. *Front. Neurosci.* **6**, 48 (2012).
22 Barbato, C., Ruberti, F. & Cogoni, C. Searching for MIND: microRNAs in neurodegenerative diseases. *BioMed Res. Int.* **2009**, 871313 (2009).
23 Selkoe, D., Mandelkow, E. & Holtzman, D. Deciphering Alzheimer disease. *Cold Spring Harb. Perspect. Med.* **2**, a011460 (2012).
24 O'Brien, R. J. & Wong, P. C. Amyloid precursor protein processing and Alzheimer's disease. *Annu. Rev. Neurosci.* **34**, 185 (2011).
25 Hernández, F. & Avila, J. The role of glycogen synthase kinase 3 in the early stages of Alzheimers' disease. *FEBS Lett.* **582**, 3848–3854 (2008).
26 Hébert, S. S., Horré, K., Nicolaï, L., Papadopoulou, A. S., Mandemakers, W., Silahtaroglu, A. N. *et al.* Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1 β-secretase expression. *Proc. Natl Acad. Sci.* **105**, 6415–6420 (2008).
27 Wang, W.-X., Huang, Q., Hu, Y., Stromberg, A. J. & Nelson, P. T. Patterns of microRNA expression in normal and early Alzheimer's disease human temporal cortex: white matter versus gray matter. *Acta Neuropathol.* **121**, 193–205 (2011).
28 Zong, Y., Wang, H., Dong, W., Quan, X., Zhu, H., Xu, Y. *et al.* miR-29c regulates BACE1 protein expression. *Brain Res.* **1395**, 108–115 (2011).
29 Zhu, H.-C., Wang, L.-M., Wang, M., Song, B., Tan, S., Teng, J.-F. *et al.* MicroRNA-195 downregulates Alzheimer's disease amyloid-β production by targeting BACE1. *Brain Res. Bull.* **88**, 596–601 (2012).
30 Fang, M., Wang, J., Zhang, X., Geng, Y., Hu, Z., Rudd, J. A. *et al.* The miR-124 regulates the expression of BACE1 β-secretase correlated with cell death in Alzheimer's disease. *Toxicol. Lett.* **209**, 94–105 (2012).
31 Nelson, P. T. & Wang, W.-X. MiR-107 is reduced in Alzheimer's disease brain neocortex: validation study. *J Alzheimer's Dis.* **21**, 75–79 (2010).
32 Yao, J., Hennessey, T., Flynt, A., Lai, E., Beal, M. F. & Lin, M. T. MicroRNA-related cofilin abnormality in Alzheimer's disease. *PLoS ONE* **5**, e15546 (2010).
33 Hébert, S. S., Sergeant, N. & Buée, L. MicroRNAs and the regulation of tau metabolism. *Int. J. Alzheimer's Dis.* **2012**, 406561 (2012).
34 Dickson, J. R., Kruse, C., Montagna, D. R., Finsen, B. & Wolfe, M. S. Alternative polyadenylation and miR-34 family members regulate tau expression. *J. Neurochem.* **127**, 739–749 (2013).
35 Shtilbans, A. & Henchcliffe, C. Biomarkers in Parkinson's disease: an update. *Curr. Opin. Neurol.* **25**, 460–465 (2012).
36 Houlden, H. & Singleton, A. B. The genetics and neuropathology of Parkinson's disease. *Acta Neuropathol.* **124**, 325–338 (2012).
37 Kim, J., Inoue, K., Ishii, J., Vanti, W. B., Voronov, S. V., Murchison, E. *et al.* A MicroRNA feedback circuit in midbrain dopamine neurons. *Science* **317**, 1220–1224 (2007).
38 Saiki, S., Sato, S. & Hattori, N. Molecular pathogenesis of Parkinson's disease: update. *J. Neurol. Neurosurg. Psychiatry* **83**, 430–437 (2011).
39 Wang, G., van der Walt, J. M., Mayhew, G., Li, Y.-J., Züchner, S., Scott, W. K. *et al.* Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of α-synuclein. *Am. J. Hum. Gen.* **82**, 283–289 (2008).
40 Mouradian, M. M. MicroRNAs in Parkinson's disease. *Neurobiol. Dis.* **46**, 279–284 (2012).
41 Doxakis, E. Post-transcriptional regulation of α-synuclein expression by mir-7 and mir-153. *J. Biol. Chem.* **285**, 12726–12734 (2010).
42 Junn, E., Lee, K.-W., Jeong, B. S., Chan, T. W., Im, J.-Y. & Mouradian, M. M. Repression of α-synuclein expression and toxicity by microRNA-7. *Proc. Natl Acad. Sci.* **106**, 13052–13057 (2009).
43 Khodr, C. E., Pedapati, J., Han, Y. & Bohn, M. C. Inclusion of a portion of the native SNCA 3′ UTR reduces toxicity of human S129A SNCA on striatal-projecting dopamine neurons in rat substantia Nigra. *Dev. Neurobiol.* **72**, 906–917 (2012).
44 Gehrke, S., Imai, Y., Sokol, N. & Lu, B. Pathogenic LRRK2 negatively regulates microRNA-mediated translational repression. *Nature* **466**, 637–641 (2010).
45 Cho, H. J., Liu, G., Jin, S. M., Parisiadou, L., Xie, C., Yu, J. *et al.* MicroRNA-205 regulates the expression of Parkinson's disease-related leucine-rich repeat kinase 2 protein. *Hum. Mol. Genet.* **22**, 608–620 (2013).
46 Conaco, C., Otto, S., Han, J.-J. & Mandel, G. Reciprocal actions of REST and a microRNA promote neuronal identity. *Proc. Natl Acad. Sci. USA* **103**, 2422–2427 (2006).

47 Siwach, P. & Ganesh, S. Tandem repeats in human disorders: mechanisms and evolution. *Front. Biosci.* **13**, 4467–4484 (2007).
48 Cattaneo, E., Zuccato, C. & Tartari, M. Normal huntingtin function: an alternative approach to Huntington's disease. *Nat. Rev. Neurosci.* **6**, 919–930 (2005).
49 Gil, J. M. & Rego, A. C. Mechanisms of neurodegeneration in Huntington's disease. *Eur. J. Neurosci.* **27**, 2803–2820 (2008).
50 Shoulson, I. & Young, A. B. Milestones in huntington disease. *Mov. Disord.* **26**, 1127–1133 (2011).
51 Savas, J. N., Makusky, A., Ottosen, S., Baillat, D., Then, F., Krainc, D. *et al.* Huntington's disease protein contributes to RNA-mediated gene silencing through association with Argonaute and P bodies. *Proc. Natl Acad. Sci.* **105**, 10820–10825 (2008).
52 Johnson, R., Zuccato, C., Belyaev, N. D., Guest, D. J., Cattaneo, E. & Buckley, N. J. A microRNA-based gene dysregulation pathway in Huntington's disease. *Neurobiol. Dis.* **29**, 438–445 (2008).
53 Lee, S.-T., Chu, K., Im, W.-S., Yoon, H.-J., Im, J.-Y., Park, J.-E. *et al.* Altered microRNA regulation in Huntington's disease models. *Exp. Neurol.* **227**, 172–179 (2011).
54 Martí, E., Pantano, L., Bañez-Coronel, M., Llorens, F., Miñones-Moyano, E., Porta, S. *et al.* A myriad of miRNA variants in control and Huntington's disease brain regions detected by massively parallel sequencing. *Nucleic Acids Res.* **38**, 7219–7235 (2010).
55 Packer, A. N., Xing, Y., Harper, S. Q., Jones, L. & Davidson, B. L. The bifunctional microRNA miR-9/miR-9* regulates REST and CoREST and is downregulated in Huntington's disease. *J. Neurosci.* **28**, 14341–14346 (2008).
56 Zuccato, C., Tartari, M., Crotti, A., Goffredo, D., Valenza, M., Conti, L. *et al.* Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nat. Genet.* **35**, 76–83 (2003).
57 Bañez-Coronel, M., Porta, S., Kagerbauer, B., Mateu-Huertas, E., Pantano, L., Ferrer, I. *et al.* A pathogenic mechanism in Huntington's disease involves small CAG-repeated RNAs with neurotoxic activity. *PLoS Genet* **8**, e1002481 (2012).
58 Sinha, M., Mukhopadhyay, S. & Bhattacharyya, N. P. Mechanism (s) of alteration of micro RNA expressions in Huntington's disease and their possible contributions to the observed cellular and molecular dysfunctions in the disease. *Neuromol. Med.* **14**, 221–243 (2012).
59 Allingham, R. R., Liu, Y. & Rhee, D. J. The genetics of primary open-angle glaucoma: a review. *Exp. Eye Res.* **88**, 837–844 (2009).
60 Weinreb, R. N., Aung, T. & Medeiros, F. A. The pathophysiology and treatment of glaucoma: a review. *JAMA* **311**, 1901–1911 (2014).
61 Dang, H. & Dang, M. M.-H. The impact of obesity and lifestyle-related risk indicators in open-angle glaucoma: a review. *Univ. Toronto Med. J.* **91**, 36–39 (2014).
62 Izzotti, A., Longobardi, M., Cartiglia, C., Rathschuler, F. & Saccà, S. C. Trabecular meshwork gene expression after selective laser trabeculoplasty. *PLoS ONE* **6**, e20110 (2011).
63 Villarreal, G., Oh, D.-J., Kang, M. H. & Rhee, D. J. Coordinated regulation of extracellular matrix synthesis by the microRNA-29 family in the trabecular meshwork. *Invest. Ophthalmol. Vis. Sci.* **52**, 3391–3397 (2011).
64 Vidal-Sanz, M., Salinas-Navarro, M., Nadal-Nicolás, F. M., Alarcón-Martínez, L., Valiente-Soriano, F. J., de Imperial, J. M. *et al.* Understanding glaucomatous damage: anatomical and functional data from ocular hypertensive rodent retinas. *Prog. Retin. Eye Res.* **31**, 1–27 (2012).
65 Izzotti, A., Ceccaroli, C., Longobardi, M. G., Micale, R. T., Pulliero, A., La Maestra, S. *et al.* Molecular damage in glaucoma: from anterior to posterior eye segment. The MicroRNA role. *MicroRNA* **4**, 3–17 (2015).
66 Colombo, M., Brittingham, R. J., Klement, J. F., Majsterek, I., Birk, D. E., Uitto, J. *et al.* Procollagen VII self-assembly depends on site-specific interactions and is promoted by cleavage of the NC2 domain with procollagen C-proteinase. *Biochemistry* **42**, 11434–11442 (2003).
67 Luna, C., Li, G., Qiu, J., Epstein, D. L. & Gonzalez, P. Role of miR-29b on the regulation of the extracellular matrix in human trabecular meshwork cells under chronic oxidative stress. *Mol. Vis.* **15**, 2488 (2009).
68 Luna, C., Li, G., Qiu, J., Epstein, D. L. & Gonzalez, P. Cross-talk between miR-29 and transforming growth factor-betas in trabecular meshwork cells. *Invest. Ophthalmol. Vis. Sci.* **52**, 3567 (2011).
69 Luna, C., Li, G., Huang, J., Qiu, J., Wu, J., Yuan, F. *et al.* Regulation of trabecular meshwork cell contraction and intraocular pressure by miR-200c. *PLoS ONE* **7**, e51688 (2012).
70 Paylakhi, S. H., Moazzeni, H., Yazdani, S., Rassouli, P., Arefian, E., Jaberi, E. *et al.* FOXC1 in human trabecular meshwork cells is involved in regulatory pathway that includes miR-204, MEIS2, and ITGβ1. *Exp. Eye Res.* **111**, 112–121 (2013).
71 Luna, C., Li, G., Qiu, J., Epstein, D. L. & Gonzalez, P. MicroRNA-24 regulates the processing of latent TGFβ during cyclic mechanical stress in human trabecular meshwork cells through direct targeting of FURIN. *J. Cell Physiol.* **226**, 1407–1414 (2011).
72 Surgucheva, I., Chidambaram, K., Willoughby, D. A. & Surguchov, A. Matrix metalloproteinase 9 expression: new regulatory elements. *J. Ocul. Biol. Dis. Infor.* **3**, 41–52 (2010).
73 Sundermeier, T. R. & Palczewski, K. The physiological impact of microRNA gene regulation in the retina. *Cell. Mol. Life Sci.* **69**, 2739–2750 (2012).
74 Lumayag, S., Haldin, C. E., Corbett, N. J., Wahlin, K. J., Cowan, C., Turturro, S. *et al.* Inactivation of the microRNA-183/96/182 cluster results in syndromic retinal degeneration. *Proc. Natl Acad. Sci.* **110**, 507–516 (2013).
75 Andreeva, K. & Cooper, N. G. MicroRNAs in the neural retina. *Int. J. Genomics* **2014**, 165897 (2014).
76 Kong, N., Lu, X. & Li, B. Downregulation of microRNA-100 protects apoptosis and promotes neuronal growth in retinal ganglion cells. *BMC. Mol. Biol.* **15**, 25 (2014).
77 Jayaram, H., Cepurna, W. O., Johnson, E. C. & Morrison, J. C. MicroRNA expression in the glaucomatous retina MicroRNA expression in the glaucomatous retina. *Invest. Ophthalmol. Vis. Sci.* **56**, 7971–7982 (2015).
78 Gao, L., Jiang, B., Lei, D., Zhou, X. & Yuan, H. Expression profiling of microRNAs in optineurin (E50K) mutant transgenic mice. *Biomed. Rep.* **4**, 193–196 (2016).
79 Chi, Z.-L., Akahori, M., Obazawa, M., Minami, M., Noda, T., Nakaya, N. *et al.* Overexpression of optineurin E50K disrupts Rab8 interaction and leads to a progressive retinal degeneration in mice. *Hum. Mol. Genet.* **19**, 2606–2615 (2010).
80 Tanaka, Y., Tsuda, S., Kunikata, H., Sato, J., Kokubun, T., Yasuda, M. *et al.* Profiles of extracellular miRNAs in the aqueous humor of glaucoma patients assessed with a microarray system. *Sci. Rep.* **4**, 5089 (2014).
81 Arora, A., Guduric-Fuchs, J., Harwood, L., Dellett, M., Cogliati, T. & Simpson, D. A. Prediction of microRNAs affecting mRNA expression during retinal development. *BMC Dev. Biol.* **10**, 1 (2010).
82 Baudet, M.-L., Zivraj, K. H., Abreu-Goodger, C., Muldal, A., Armisen, J., Blenkiron, C. *et al.* miR-124 acts through CoREST to control onset of Sema3A sensitivity in navigating retinal growth cones. *Nat. Neurosci.* **15**, 29–38 (2012).
83 Li, G., Luna, C., Qiu, J., Epstein, D. L. & Gonzalez, P. Modulation of inflammatory markers by miR-146a during replicative senescence in trabecular meshwork cells. *Invest. Ophthalmol. Vis. Sci.* **51**, 2976–2985 (2010).
84 Alzheimer's Association and others. 2014 Alzheimer's disease facts and figures. *Alzheimers Dement.* **10**, 47–92 (2014).
85 Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M. *et al.* Global prevalence of dementia: a Delphi consensus study. *Lancet* **366**, 2112–2117 (2006).
86 Quigley, H. A. & Broman, A. T. The number of people with glaucoma worldwide in 2010 and 2020. *Br. J. Ophthalmol.* **90**, 262–267 (2006).
87 Junn, E. & Mouradian, M. M. MicroRNAs in neurodegenerative diseases and their therapeutic potential. *Pharmacol. Ther.* **133**, 142–150 (2012).
88 Roshan, R., Ghosh, T., Scaria, V. & Pillai, B. MicroRNAs: novel therapeutic targets in neurodegenerative diseases. *Drug Discov. Today* **14**, 1123–1129 (2009).
89 Ørom, U. A., Kauppinen, S. & Lund, A. H. LNA-modified oligonucleotides mediate specific inhibition of microRNA function. *Gene* **372**, 137–141 (2006).
90 Bilen, J., Liu, N., Burnett, B. G., Pittman, R. N. & Bonini, N. M. MicroRNA pathways modulate polyglutamine-induced neurodegeneration. *Mol. Cell* **24**, 157–163 (2006).
91 Conde, J. & Artzi, N. Are RNAi and miRNA therapeutics truly dead? *Trends Biotechnol.* **33**, 141–144 (2015).
92 Conde, J., Edelman, E. R. & Artzi, N. Target-responsive DNA/RNA nanomaterials for microRNA sensing and inhibition: the jack-of-all-trades in cancer nanotheranostics? *Adv. Drug Deliv. Rev.* **81**, 169–183 (2015).
93 Höbel, S. & Aigner, A. Polyethylenimines for siRNA and miRNA delivery in vivo. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **5**, 484–501 (2013).
94 Patel, N., Hoang, D., Miller, N., Ansaloni, S., Huang, Q., Rogers, J. T. *et al.* MicroRNAs can regulate human APP levels. *Mol. Neurodegener.* **3**, 470 (2008).
95 Liu, W., Liu, C., Zhu, J., Shu, P., Yin, B., Gong, Y. *et al.* MicroRNA-16 targets amyloid precursor protein to potentially modulate Alzheimer's-associated pathogenesis in SAMP8 mice. *Neurobiol. Aging* **33**, 522–534 (2012).
96 Long, J. M. & Lahiri, D. K. MicroRNA-101 downregulates Alzheimer's amyloid-β precursor protein levels in human cell cultures and is differentially expressed. *Biochem. Biophys. Res. Commun.* **404**, 889–895 (2011).
97 Delay, C., Calon, F., Mathews, P. & Hébert, S. S. Alzheimer-specific variants in the 3'UTR of Amyloid precursor protein affect microRNA function. *Mol. Neurodegener.* **6**, 70 (2011).
98 Boissonneault, V., Plante, I., Rivest, S. & Provost, P. MicroRNA-298 and microRNA-328 regulate expression of mouse beta-amyloid precursor protein-converting enzyme 1. *J. Biol. Chem.* **284**, 1971–1981 (2009).