Circicular and long non-coding RNAs and their role in ophthalmologic diseases

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ABSTRACT

Long non-coding RNAs are >200-nucleotide-long RNA molecules which lack or have limited protein-coding potential. They can regulate protein formation through several different mechanisms. Similarly, circular RNAs are reported to play a critical role in post-transcriptional gene regulation. Changes in the expression pattern of these molecules are known to underlie various diseases, including cancer, cardiovascular, neurological and immunological disorders (Rinn & Chang, 2012; Sun & Kraus, 2015). Recent studies suggest that they are differentially expressed both in healthy ocular tissues as well as in eye pathologies, such as neovascularization, proliferative vitreoretinopathy, glaucoma, cataract, ocular malignancy or even strabismus (Li et al., 2016). Aetiology of ocular diseases is multifactorial and combines genetic and environmental factors, including epigenetic and non-coding RNAs. In addition, disorders like diabetic retinopathy or age-related macular degeneration lack biomarkers for early detection as well as effective treatment methods that would allow controlling the disease progression at its early stages. The newly discovered non-coding RNAs seem to be the ideal candidates for novel molecular markers and therapeutic strategies. In this review, we summarized the current knowledge about gene expression regulators – long non-coding and circular RNA molecules in eye diseases.

Key words: ophthalmologic diseases, neovascularization, retinopathy, AMD, ocular malignancy, long non-coding RNAs, circRNAs

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INTRODUCTION

Functional regulation of gene expression at the epigenetic, transcriptional and post-transcriptional stage has recently been thoroughly analyzed. It is well established that changes in the long non-coding RNA (IncRNA) and circular RNA (circRNA) levels are associated with the occurrence of multiple disorders, including ocular diseases. Both IncRNAs and circRNAs are relatively newly discovered molecules, but with other non-coding RNA molecules and proteins, they form a network of interactions regulating all cellular processes (Sun & Kraus, 2015; Tan, 2014; Zhong et al., 2018). Ophthalmological diseases constitute a huge group of various multifactorial disorders, often associated with other systemic diseases. Recently, it was proven that epigenetics and non-coding RNAs must also be taken into account as key players in their development. Here we recapitulate the current data about IncRNAs and circRNAs in ophthalmology and exhibit their potential as molecular markers and therapeutic targets.

CIRCULAR AND LONG NON-CODING RNAs

IncRNAs are defined as RNA transcripts with little or no coding potential (Sun & Kraus, 2015). They are longer than 200 nucleotides (nt) and were recently shown to be involved in numerous cellular processes ranging from pluripotency of embryonic stem cells, cell-cycle regulation, to the development of cancer, and other diseases. IncRNAs force the formation of ribonucleoprotein complexes, which in turn impact the regulation of gene expression (Rinn & Chang, 2012). According to the genome location, morphology, sequence, structure, and function features, IncRNAs can be categorized into different groups (Wang et al., 2017a). They can be detected in areas separated from genes encoding known protein-coding transcripts (IncRNAs – long intergenic RNAs) as well as inside protein-coding genes. Genic IncRNAs are situated in exonic or intronic regions and...
In RNA and non-coding RNAs, lncRNAs present the mechanisms of action. They regulate expression at the post-transcriptional level by "sponging" miRNA or interacting with RNA-binding proteins (Long et al., 2017). Cytoplasmic lncRNAs can influence expression of these molecules. Nuclear lncRNAs can be involved in epigenetic processes (Kraus, 2015). The biogenesis of lncRNAs, like mRNAs, occurs in the nucleus and depends on RNA Polymerase II and III. lncRNA promoters coincide with epigenetic modifications that regulate transcription factor binding in order to favour or diminish gene expression (Beermann et al., 2016). Post-transcriptional processing of lncRNAs also shares similar modifications with mRNAs including 5'-capping (Ayup et al., 2015), 3'-polyadenylation, canonical and alternative splicing events and RNA editing processes (Bond & Fox, 2009; Khandelwal et al., 2015). Interestingly, it was observed that lncRNAs are capable of forming secondary structures based on base-pairing or ribose backbone interactions that determine the final function of the molecule (Beermann et al., 2016; Mercer et al., 2009). High-throughput sequencing of lncRNAs showed that they contain modified bases that also impact their structure and function (Kellner et al., 2010). In post-transcriptional lncRNA modifications can be reversible which confirms that the functional regulation of these molecules is highly complex (Mercer &Mattick, 2013).

Gene regulation by lncRNAs occurs at many different levels, through nuclear and cytoplasmic mechanisms. The cellular localization of a lncRNA can indicate its mode of action. Nuclear lncRNAs can be involved in histone modification or direct transcriptional regulation. Cytoplasmic lncRNAs regulate expression at the post-transcriptional level by "sponging" miRNA or interacting with RNA-binding proteins (Long et al., 2017). Figure 1 presents the mechanisms of action of long non-coding RNAs.

InRNA expression patterns can impact cell functions which manifests as a pathological process in disease. Profiling of lncRNAs revealed differences in their expression between normal and carcinogenic cells (Rasool et al., 2016) and they were recognized as having oncogenic and suppressive roles in neoplasia (Yan & Wang, 2012). For example, down-regulation of H19, a widely known lncRNA having a role in cancerogenesis, reduces the growth of breast and lung cancers (Chen et al., 2017b; Tessier et al., 2004). In bladder cancer, the up-regulation of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is associated with the blocking of apoptosis and enhancing cancer cell proliferation and migration (Taberi et al., 2018). lncRNAs are involved in regulating the ageing process, thereby contributing to the development of age-related diseases such as obesity, diabetes, and neurodegeneration. One of the previously mentioned lncRNAs, H19, was reported to control imprinting of various genes, including insulin-like growth factor 2, and to be involved in fat metabolism and deposition (Kim et al., 2016). Many lncRNAs play a role in the regulation of gene expression in the central nervous system. They were described to contribute to synapse formation, maturation of neurons, oligodendrocytes and neuronal-glial fate transition as well as to the regulation of hippocampal development (Mercer et al., 2010). An interesting example of disorders involving lncRNAs is Fragile X syndrome (FXS), a heritable mental disorder caused by expansion of triplet nucleotide repeats in FMR1 gene encoding FMRP – neuronal development protein. It was reported that a vast majority of lncRNAs, which may play a role in FXS, originate from FMR1 gene (Pastori & Wahlestedt, 2012). There are many other disorders triggered by lncRNA dysregulation, such as rheumatic diseases (Tang et al., 2017), cardiovascular diseases (Sallam et al., 2018) and autoimmune diseases. The regulatory networks in various disorders where gene expression patterns are disturbed often involve lncRNA-mRNA interactions and still remain to be precisely characterized in order to better understand the pathomechanisms (Zhang et al., 2017d).

Circular RNAs (circRNAs) are non-coding and ubiquitously expressed RNAs, which has been broadly studied in recent years also can serve as useful markers in ocular diseases. circRNA expression patterns are disturbed often involve lncRNA-mRNA interactions and still remain to be precisely characterized. Most exonic circRNAs occur in the cytoplasm, whereas the other two are mainly found in the nucleus (Memczak et al., 2013). The first circRNA transcripts were identified in the early 1990s, but a breakthrough in circRNA research occurred in 2013 after the Salzman’s group provided evidence that certain human transcripts prefer a circular form rather than linear (Salzman et al., 2012). circRNAs can arise from virtually any part of the genome (exonic and non-coding, transcripts antisense to 5' and 3' UTRs or intergenic regions) which causes significant differences in the length of molecules (Rong et al., 2017). CircRNAs are considered to be ubiquitous and evolutionarily conserved among species, suggesting a significant regulatory role.

Biogenesis of circular transcripts is a highly regulated process. Covalently closed RNA molecules might appear as the result of direct RNA ligation, circularization of introns which escaped from debranching or may derive from the intermediates of processed RNAs. Howev-
er, the large majority are generated from pre-messenger RNA (pre-mRNA) in the back-splicing process (Wang & Wang, 2015). Back-splicing is the more advanced type of splicing, however, circRNA that cannot be formed in the canonical manner still requires the presence of canonical spliceosomal machinery and signals (Chen & Yang, 2015). In contrast to canonical splicing, where an upstream 5’ splice donor site binds to the downstream 3’ splice acceptor site in a sequential order to generate the linear transcripts, back-splicing involves reverse orientation that links a downstream 5’ splice donor site to an upstream 3’ splice acceptor resulting in exons in a reversed order (Zhang et al., 2014).

The biogenesis of circRNA also depends on the location of the sequence within the genome. Hence, two general factors promoting the circularization are known – cis-elements and trans-factors-dependent. Circular RNAs classification is based on their origin, taking into account the contribution of cis-elements: exonic, exon-intron (EIciRNAs) and intronic molecules (Jeck et al., 2013). However, Jeck et al. provided evidence (2013) that nearly all circRNAs comprise of exonic sequences of protein-coding genes, formed from one or multiple exons, most frequently 1-5 (Memczak et al., 2013). A variety of RNA-binding proteins – trans-factors also has a significant role in the production of circRNA. Muscleblind (MBL/MBNL1) splicing factor promotes the circularization of certain transcripts. Moreover, Ashwal-Fluss and coworkers in 2014 (Ashwal-Fluss et al., 2014) described the circular form of Muscleblind (circMBL) in flies and humans, resulting from the circularization of the second exon. They discovered putative MBL binding sites present within the intronic regions flanking the second exon. This led to the conclusion that certain MBL isoforms might promote their own exon circularization. Indeed, the level of circMBL was decreased after knockdown of endogenous MBL, demonstrating its function as a circMBL promoting factor (Ashwal-Fluss et al., 2014). The following example of binding elements, adenosine deaminases acting on RNA – ADARs, which are known to convert adenosine to inosine in double-stranded RNA, were reported to affect the circRNAs biogenesis in a negative manner. It is suggested that negative regulation is associated with their function – adenosine to inosine (A-to-I) RNA editing. High level of A-to-I editing, double-stranded RNA is known to deplete RNA pairing, which results in diminished pairing and closing of the ends, while lower levels of ADAR promote more stable pairing across the introns and back-splicing for circular RNA production (Chen & Yang, 2015). The abundance of circRNAs in humans is also regulated by Quaking (QKI) – RNA binding protein, which mediates exon circularization by binding at up- and downstream position of circRNA-forming exons and dimerization coupled with bringing 3’ and 5’ ends of the exon in close proximity which results in joining (Salzman, 2016). Intriguingly, the circularization of linear transcripts is possible after the insertion of Quaking binding sites into the flanking region of linear RNAs (Conn et al., 2015). Even though circular RNA molecules are a relatively new class of long, non-coding RNAs, extensive research has been carried out to determine the function they perform. Many circRNAs are currently considered as regulatory molecules, particularly affecting the function of microRNAs (miRNA), due to the presence of a number of binding sites allowing the interaction to occur. This type of relationship results in circRNA-mediated repression of miRNA function, where endogenous circular transcripts work as miRNA sponges. It was shown that conserved miRNA and AGO protein binding sites are enriched in circRNAs. For at least one specific circRNA, dRS-75 (also called CDR1as), has more than 70 binding sites for miR-7. It was shown in vivo that this circRNA impairs the regulatory role of miR-7. However, whether or not all circRNAs function as miRNA sponges is still not clear. An additional function of circRNAs is the transport of miRNAs. However, available experimental data are not very comprehensive especially for their global regulation and function (Memczak et al., 2013). Apart from the function as miRNA sponges, circRNAs were shown to regulate alternative splicing and to modulate the expression of parental genes (Guo et al., 2014; Salzman et al., 2013; Salzman et al., 2012; Zhang et al., 2013). Recently, circRNAs were studied in relation to their role in the translation of proteins or peptides (Pamudurti et al., 2017). Four mechanisms of circRNA protein or peptide translation have been identified up to now: (1) involving internal ribosome entry sites (IRESs) within synthetic circRNA; (2) effective circRNA translation via rolling circle amplification (RCA); (3) translation driven by N6-methyladenosine (m6A) and (4) a novel cap-independent translation mechanism (Li et al., 2017c; Wang & Wang, 2015; Yang et al., 2017). More importantly, it is becoming evident that circRNAs may be involved in many types of non-neoplastic diseases, such as e.g. atherosclerotic vascular disease risk, neurological disorders, prion and Alzheimer diseases, rheumatoid arthritis or kidney injury. circRNAs can play a crucial role in tumorigenic processes in different types of cancers, such as: ovarian carcinoma, bladder, papillary thyroid, colorectal, lung or breast cancers (Hu et al., 2018b; Ren et al., 2018; Wang et al., 2018b; Yang et al., 2018a; Zhang et al., 2018). circRNAs were described also as potential disease biomarkers in human saliva and blood and as biomarkers for ageing and gastric cancer (Bachmayr-Heyda et al., 2014).
non-coding RNAs. *Vax2os1* is a retina-specific lncRNA that regulates the cell cycle in photoreceptor progenitor cells. Disturbances in its expression pattern result in the delay of the differentiation process (Meola et al., 2012). *TUG 1* is another lncRNA found in developing retina. It is responsible for the formation of photoreceptors by activation of specific genes and is also expressed in endothelial cells during ROP (Michalik et al., 2014; Young et al., 2005). Bioinformatics analysis performed by Yang et al. showed several circular and long non-coding RNAs involved in ROP development and progression. They correlated the expression of lncRNAs POLDIP2, GA53, NEFL and UHRF1 with miRNA-128-3p and miRNA-9-3p levels which significantly differ between retinas of neonatal mice and rats with and without ROP. Similarly, circRNA ZNF290C_hsa_circ_0012711 and SIAE_hsa_circ_002083 are also differentially expressed in ROP. Their exact function is still unclear, but the authors implied an association with TGFβ and PI3K-Akt signalling pathways responsible for cell migration and angiogenesis (Yang et al., 2018c). In *in vitro* and *in vivo* studies of lncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) revealed that it regulates angiogenesis in developing retina as well as in disorders related to hypoxia. Silencing *MALAT1* expression by siRNA resulted in a reduction of total *MALAT1* levels, both cytoplasmic and nuclear and increased basal endothelial cell migration, whereas cell proliferation was down-regulated. Therefore, inhibition of this lncRNA may become a potential therapeutic strategy for pathological retinal neovascularization (Michalik et al., 2014).

**Neovascularization**

Formation of new blood vessels is a process necessary for proper development, but under pathological conditions such as tissue damage or hypoxia abnormal vascular growth occurs. Neovascularization is associated with a number of ocular disorders including corneal injury or infection, retinopathy of prematurity (ROP), age-related macular degeneration (AMD) and proliferative diabetic retinopathy (PDR) (Xu et al., 2014; Zhang et al., 2017c). In all of these conditions the genetic background plays a significant role, however, it does not fully explain the diversity of the clinical picture. Participation of lncRNAs and circRNAs in most biological processes, including cell differentiation, proliferation, and apoptosis, can describe the relationship between gene expression and environmental factors affecting the progress of these diseases (Yan et al., 2014).

**Corneal neovascularization**

The causes of corneal neovascularization (CN) include infections, injuries, and graft rejections. The process is strictly controlled by two counterbalancing systems: stimulators and inhibitors of angiogenesis, and can lead to severe vision loss or even blindness (Huang et al., 2015). Huang et al. provided evidence that lncRNAs are potential regulators of CN pathogenesis. They identified 154 lncRNAs differentially expressed between normal and vascularized corneas of which 60 were down-regulated, and 94 were up-regulated. Expression patterns of randomly selected lncRNAs were also compared to the patterns of antiangiogenic factors – Platelet-Derived Growth Factor (PDGF) and endostatin, and proangiogenic factors – Vascular Endothelial Growth Factor (VEGF) and Nitric Oxide Synthases isoform (iNOS). For example, the human or- tholog of *Vax2* was significantly up-regulated in the vascularized corneas and demonstrated similar expression profile as VEGF. By contrast, the human ortholog of lincRNA: chr8:129102060–129109035 reverse strand, was markedly down-regulated and resembled expression by siRNA resulting of *Vax2*. Thus, it is demonstrated that lncRNAs can become potential targets for their prevention or treatment (Lī et al., 2016).

**Retinopathy of Prematurity**

Retinopathy of prematurity (ROP) occurs in premature neonates, in whom at the time of birth the retina remains incompletely vascularized. Instead of proper vascular development, vasculogenesis in the premature neonatal retina is disrupted. At the border of the avascular retina, abnormal vessels grow into the vitreous resulting in haemorrhage and tractional detachment of the retina (Neely & Gardner, 1998). Development of the retina and its vasculization is a complicated process, and it depends on many factors, which include long
Dry AMD progression is slower than wet AMD and less likely leads to loss of the central visual field. It also causes severe visual impairment, especially in the advanced form of the disease called geographic atrophy which can develop in areas of regressed large drusen but also independently, in areas of prior pigmentary changes suggesting RPE dysfunction (Yonekawa et al., 2015). Early AMD was reported to be influenced by oxidative stress, abnormal lipid metabolism, cell apoptosis and dysfunction of the immune system, whereas IncRNAs are also associated with these processes (Sun & Kraus, 2015). Zhu et al. found 64 IncRNAs dysregulated in early AMD and established that they could play an important role in AMD development. Mapping of IncRNA-related dysregulated miRNAs showed that most of them locate in phototransduction and purine metabolism pathways. They evaluated the expression level of one IncRNA – RP11-23406.2 in *in vivo* and studied its activity in the aging model of cultured retinal pigmented epithelium (ARPE-19) cells. RP-1123406.2 was downregulated in early AMD, and its exogenous application improved cell viability and reduced the rate of early apoptosis (Zhu et al., 2017). ZNF503-AS1 is an intergenic IncRNA up-regulated during RPE differentiation and down-regulated in RPE-choroid of atrophic AMD. It can potentially promote RPE differentiation through inhibiting its target – ZNF503. ZNF503-AS1 is regulated by NF-xB which is involved in cellular processes such as inflammation and differentiation of RPE. Targeting both RP-1123406.2 and ZNF503-AS1 is a potential therapeutic strategy for atrophic AMD (Chen et al., 2017a).

**Diabetic retinopathy**

Diabetic retinopathy (DR) is one of the most common vascular complications in patients with long-term diabetes. Changes in microvascular circulation in the retina under hyperglycaemia conditions include increased proliferation and permeability of endothelial cells, abnormal neovascularization, and edema. Elevated blood glucose level results in oxidative stress, inflammation, neuronal dysfunction, apoptosis of the retinal ganglion cells and activation of glial cells. In all of these processes, IncRNAs play a significant role by interacting with chemokine and mitogen-activated protein kinase (MAPK) signalling pathways (Gong & Su, 2017; Pradhan et al., 2016).

Myocardial infarction associated transcript (MIAT) is a retinal IncRNA 2 (RNCR2) that is highly expressed in retinal precursor cells and the retinas of diabetic rats. Its expression is also observed in Müller cells isolated from diabetic mice. Apoptotic activity is associated with the interaction with NF-xB (heterodimer that comprises p65 and p50) which selectively binds to the MIAT promoter. MIAT silencing under diabetic conditions inhibits apoptosis and improves visual functions (Gong & Su, 2017; Zhang et al., 2017a).

Another IncRNA involved in the regulation of epithelial cell function is MAL-AT1. Its cooperation with p38 MAPK signalling pathway affects cell proliferation, migration, and tube formation. Knockdown of MAL-AT1 in cell lines induces a change of phenotype from proliferative to migratory, and *in vivo* silencing reduces vascular growth (Michalk et al., 2014). Diabetic retinopathy progression is influenced by a complex crosstalk between angiogenesis and inflammation. MAL-AT1 also participates in inflammatory activation. Under hyperglycemic conditions, it is upregulated in human retinal endothelial cells (HRECs). Its siRNA silencing reduces the expression of several inflammatory cytokines, including tumour necrosis factor-alpha (TNFα), interleukin-6 (IL-6), interleukin-1β (IL-1β) and monocyte chemotactic protein-1 (MCP-1). Also, the histone methyltransferase component of polycomb repressive complex 2 (PRC2) was downregulated in HERCs treated with siMALAT1, demonstrating its ability to influence the expression on the protein level. Reduction of TNFα, IL-6, IL-1β and MCP1 levels in *MALAT1* knockout mice with induced diabetes confirmed the importance of MALAT1 genes in the regulation of inflammation (Biswas et al., 2018). MALAT1 also promotes the inflammatory reaction in microglial cells by ‘sponging’ miR-124. Overexpression of MALAT1 in Amadori-glycated albumin (AGA) treated microglial cells results in downregulation of miR-124 which in turn leads to MCP-1 upregulation (Dong et al., 2018). ME3 can be responsible for enhanced retinal vessel dysfunction, capillary degeneration, increased epithelial permeability, and inflammation. It is significantly downregulated in diabetic mice retinas and re-establishing its expression may serve as a therapeutic strategy in diabetes-related vascular complications (Qiu et al., 2016).

SOX2 overlapping transcript (SOX2OT) and retinal ncrna3 (RNCR3) are IncRNAs also dysregulated under hyperglycemic conditions. SOX2OT is involved in pathways of apoptosis and cell viability connected with transcription factor NRF2 and its target – HO-1 gene. It is downregulated in retinal ganglion cell lines exposed to high glucose levels and oxidative stress and in the retina of diabetic mice (Li et al., 2017a). RNCR3 participates in the retinal development and neuronal and oligodendrocyte differentiation. It is also up-regulated in RF/6A cell line and the retina of diabetic mice, similarly to some interleukins and inflammatory factors like VEGF and TNFα (Liu et al., 2016; Rapicavoli et al., 2010).

Interaction of circular RNAs with miRNAs involved in proliferative and apoptotic pathways may also affect the retinal vascular dysfunction. Zhang et al. identified 529 circRNAs differentially expressed between diabetic and non-diabetic retinas. They thoroughly analyzed circ_0005015 expression profile and confirmed it is up-regulated in the plasma, vitreous samples and fibrovascular membranes of diabetic patients. circ_0005015 regulates retinal endothelial cell proliferation, migration, and tube formation. MMP-2, XIAP, and STAT3 are proteins involved in regulation of cell cycle, proliferation, and apoptosis. circ_0005015 acts as a sponge for miR-519d-3p inhibiting its activity and interfering with MMP-2, XIAP, and STAT3 expression (Zhang et al., 2017b).

The circHIPK3 expression is also up-regulated in retinal endothelial cells exposed to high glucose concentration. It acts as an endogenous miR-30a-3p sponge. Similarly to circ_0005015/miR-519d-3p, it effectively up-regulates vascular endothelial growth factors expression and intensifies endothelial proliferation, vascular leakage, and inflammation (Shan et al., 2017).

**Proliferative vitreoretinopathy**

Proliferative vitreoretinopathy (PVR) is a serious complication of retinal detachment and vitreoretinal surgery. It can lead to severe vision reduction due to retinal re-detachment caused by the formation of preretinal and epiretinal membrane (ERM) traction. Several cell types are associated with the PVR pathogenesis, including retinal pigment epithelial (RPE) cells, fibroblasts, glial cells, and inflammatory cells. RPE cells are the largest cellular component in epiretinal membranes, and, importantly, they undergo dedifferentiation process – epithelial-mesenchymal transition (EMT) – in which they acquire...
a mesenchymal phenotype (Kaneko & Terasaki, 2017; Yang et al., 2016). This process is the main contributor to PVR progression and involves a number of molecular pathways affecting cell proliferation and migration. Zhou et al. established that 78 lncRNAs were abnormally expressed in ERMs of PVR patients. They focused on MALAT1 whose up-regulation contributed to RPE cell proliferation, migration, and epithelial-mesenchymal transition. It was also found that its level in peripheral blood samples of the patients differs before and after PVR surgical treatment. This evidence suggests MALAT1 could be used for the diagnosis and monitoring of PVR progression (Wan et al., 2017; Zhou et al., 2015).

Yang and coworkers (Yang et al., 2016) confirmed the role of MALAT1 in PVR pathogenesis. They reported it is involved in EMT upon transforming growth factor β1 (TGFβ1) induction. Knock-down of MALAT1 by specific siRNA resulted in TGFβ1-induced morphological change inversion, suppression of migration and proliferation of RPE cells.

Glaucoma

Glaucoma is the leading cause of irreversible vision loss. It is a group of eye diseases characterized by retinal neurodegeneration comprising retinal ganglion cell loss, optic disc excavation that results in progressive loss of visual field (Abu-Amero et al., 2015). The most common type of glaucoma is primary open angle glaucoma (POAG) in which the anterior iridocorneal chamber angle is opened. The condition is often associated with increased intraocular pressure (IOP), but can also occur with normal IOP – normal tension glaucoma (NTG). Glaucoma is a multifactorial disorder with genetic and epigenetic components (Abu-Amero et al., 2015; Gauthier & Lau, 2017).

The cyclin-dependent kinase inhibitor 2B antisense non-coding RNA (CDKN2B-AS1) also known as ANRIL is a lncRNA transcribed in an antisense direction, located on chromosome 9p21. Several studies showed that ANRIL is associated with POAG, but its effects are not thoroughly understood. The genotype/phenotype analysis revealed a significant correlation between CDKN2B-AS1 and decreased intraocular pressure in POAG patients. It suggests that ANRIL modifies the vulnerability of the optic nerve and modulates neurodegeneration. Patients carrying the risk alleles in ANRIL region are predisposed to develop POAG at lower IOP levels and to exhibit normal tension glaucoma (Nakano et al., 2012; Shiga et al., 2017).

Wang et al. investigated ZRANB1 – a circRNA significantly upregulated in glaucoma-induced retinal neurodegeneration. It is mainly expressed in the cytoplasm of glial cells, implying a regulatory activity at the post-transcriptional level. It also may suppress the expression of miR-217 resulting in increased Müller cells proliferation. ZRANB1 knock-down decreases retinal reactive gliosis and reduces glaucoma-induced retinal ganglion cell apoptosis. Additionally, they established that overexpression of transcription factor RUNX2 reverses ZRANB1 knockdown effects. The ZRANB1/miR-217/RUNX2 signalling network is a potential therapeutic target for treating retinal neurodegeneration (Wang et al., 2018a).

Extensive proliferation of glaucoma Tenon’s capsule fibroblasts (GTf) and subsequent scarring is the main cause of glaucoma filtration surgery (GFS) failure. Transforming growth factor β2 (TGFβ2) is upregulated after GFS surgery. It regulates proliferative ability, apoptosis, and differentiation of fibroblasts. TGFβ2 acts via different molecular pathways, including interacting with nuclear factor 2 (Nrf2), which is involved in retinal ganglion cells apoptosis and fibroblasts proliferation. Wang et al. investigated the role of MEG3 lncRNA in the TGFβ2-stimulated proliferation of fibroblasts. The overexpression of MEG3 was correlated with Nrf2 up-regulation. Their possible direct interaction causes a synergistic effect of reduced GTF proliferation, suggesting that MEG3 may act as a therapeutic tool for improving glaucoma filtration surgeries (Wang et al., 2017b).

TGFβ is also involved in lncRNA Growth Arrest Specific 5 (GAS5) pathway. Retinal ganglion cells (RGC) transfected with siRNA targeting GAS5 showed increased proliferation and differentiation. The axon length was significantly improved in GAS5-low-expression group compared to the control group. These results revealed that high expression of lncRNA GAS5 may promote glaucoma progression and ganglion cell degeneration. RGCs treated with exogenously administrated TGFβ demonstrated decreased GAS5 levels in a time- and dose-dependent manner, indicating its protective potential in glaucoma neurodegeneration (Xu & Xing, 2018).

OTHER OPHTHALMOLOGIC DISORDERS

Ocular malignancy

The expression of lncRNAs in cancerogenesis is well established. Du and coworkers (Du et al., 2013) performed a global analysis of more than 10,000 lncRNA genes in 1,300 tumour samples of different cancer types. They determined lncRNAs are associated with cellular oncogenic potential and promote metastasis but may also act as tumour suppressors (Du et al., 2013; Sun & Kraus, 2015).

There are several lncRNAs related to uveal melanoma. CRNDE is a lncRNA that promotes cell proliferation and invasion through the mTOR signalling pathway and modulates the methylation status of histones. Mutation SF3B1 related to the alternative splicing of CRNDE genes is associated with good prognosis in patients with uveal melanoma (Furney et al., 2013). Another lncRNA – LINC-ROR is up-regulated in uveal melanoma cell lines and tumour specimens. By repelling the histone methyltransferase EHMT2 (also known as G9a), it activates the TET2 promoter and causes an oncogenic effect (Fan et al., 2015; Wan et al., 2017).

Robertson and coworkers (Robertson et al., 2017) provided a comprehensive multiplatform analysis of 80 uveal melanomas. They identified four molecularly distinct tumour subtypes, two associated with poor prognosis (monosomy of chromosome 3 – M3) and two with better prognosis (dismy of chromosome 3 – D3). Investigating the expression profile of 8,167 lncRNAs, they noted that well-established cancer-associated LINC00152 (CYTOR) and BANCR are overexpressed in poor-prognosis subgroups. The upregulation of LINC00152 and BANCR correlates with invasion, cell migration and proliferation (Robertson et al., 2017). Also, lncRNA PTET localized in well-known cancer risk region 8q24, was among the most differentially expressed transcripts in poor-prognosis groups. Its oncogenic potential is associated with MYC transcription factors. PTET controls MYC expression at the post-transcriptional level by increasing the protein’s stability and promotes cell proliferation (Colombo et al., 2015). The quantity of PTET,
Table 1. Long non-coding RNAs and circular RNAs in ophthalmologic disorders.

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<th>IncRNA</th>
<th>Dysregulation</th>
<th>Function</th>
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<td>Regulates angiogenesis in developing retina</td>
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<td>Affects cell proliferation, migration, and tube formation, interacts with p38 MAPK</td>
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<td>MEG3</td>
<td>Down-regulated</td>
<td>Enhances retinal vessel dysfunction</td>
<td>Qiu et al., 2016</td>
</tr>
<tr>
<td></td>
<td>SOX2OT</td>
<td>Down-regulated</td>
<td>Promotes neurodegeneration, apoptosis, affects cell viability</td>
<td>Li et al., 2017a</td>
</tr>
<tr>
<td>Diabetic Retinopathy</td>
<td>RNCRI3</td>
<td>Up-regulated</td>
<td>Increases cell viability, proliferation, and migration</td>
<td>Liu et al., 2016</td>
</tr>
<tr>
<td></td>
<td>circ_0005015</td>
<td>Up-regulated</td>
<td>Acts as a miR-519d-3p sponge, regulates endothelial cell proliferation, migration and tube formation</td>
<td>Zhang et al., 2017b</td>
</tr>
<tr>
<td></td>
<td>circHIPK3</td>
<td>Up-regulated</td>
<td>Acts as miR-30a-3p sponge, intensifies endothelial proliferation, vascular leakage, and inflammation</td>
<td>Shan et al., 2017</td>
</tr>
<tr>
<td>Proliferative Vitreoretinopathy</td>
<td>MALAT1</td>
<td>Up-regulated</td>
<td>Promotes epithelial-mesenchymal transition</td>
<td>Yang et al., 2016</td>
</tr>
<tr>
<td>Primary Open Angle Glaucoma</td>
<td>CDKN2B/AS1/ANRIL</td>
<td>Up-regulated</td>
<td>Modifies the vulnerability of the optic nerve and modulates neurodegeneration</td>
<td>Nakano et al., 2012; Shiga et al., 2017</td>
</tr>
<tr>
<td></td>
<td>cZRANBI</td>
<td>Up-regulated</td>
<td>Suppresses the expression of miR-217, increases Müller cell proliferation</td>
<td>Wang et al., 2018a</td>
</tr>
<tr>
<td>Uveal melanoma</td>
<td>LINC-ROR</td>
<td>Up-regulated</td>
<td>Induces pro-oncogenic effect</td>
<td>Fan et al., 2015</td>
</tr>
<tr>
<td></td>
<td>LINC00152</td>
<td>Up-regulated</td>
<td>Induces proliferation, cell invasion, and migration</td>
<td>Robertson et al., 2017</td>
</tr>
</tbody>
</table>
as well as LINC00152, was significantly dependent on DNA methylation.

**CYSLTR2** may act as a better prognostic marker of uveal melanoma as its expression was markedly lower in the D3 molecular subtype of the tumour (Robertson et al., 2017).

Retinoblastoma is an embryonic malignant tumour that arises from foetal stem cells in the nuclear layer of the retina. It is the most frequent primary intraocular malignancy in children. MEG3 is a potential therapeutic target and disease-specific marker for early diagnosis of this tumour. It is down-regulated in retinoblastoma samples, and its levels are associated with the stages of cancer. The MEG3 level in early-stage patients was significantly higher than in advanced-stage patients and correlated with nodal or distant metastasis. Its down-regulation correlates with progression and poor prognosis in retinoblastoma and is an independent marker for predicting the clinical outcome of retinoblastoma patients (Gao & Lu, 2016).

**AFAP1-AS1** overexpression is strongly correlated with tumour size, optic nerve invasion, and choroidal invasion. Knockdown of AFAP1-AS1 decreased cell proliferation, migration, and invasion and blocked cell cycle progression (Hao et al., 2018). Similarly, HOTAIR (HOX antisense intergenic RNA) high expression was noticed in retinoblastoma tumours bigger than 10 mm, bilateral and with lymph nodal metastasis. Contrarily, miR-613 was significantly down-regulated in the same samples. 

<table>
<thead>
<tr>
<th>LncRNA</th>
<th>Regulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BANCR</td>
<td>Up-regulated</td>
<td>Induces proliferation, cell invasion and migration</td>
</tr>
<tr>
<td>PVT1</td>
<td>Up-regulated</td>
<td>Stabilization of MYC protein, cooperation with MYC protein</td>
</tr>
<tr>
<td>MEG3</td>
<td>Down-regulated</td>
<td>Early stages marker</td>
</tr>
<tr>
<td>BANCR</td>
<td>Up-regulated</td>
<td>Promotes choroidal invasion and optic nerve invasion</td>
</tr>
<tr>
<td>AFAP1-AS1</td>
<td>Up-regulated</td>
<td>Induces proliferation, cell migration and invasion</td>
</tr>
<tr>
<td>HOTAIR</td>
<td>Up-regulated</td>
<td>Induces proliferation, cell migration and invasion Targets miR-613</td>
</tr>
<tr>
<td>H19</td>
<td>Up-regulated</td>
<td>Promotes proliferation, cell migration and invasion</td>
</tr>
<tr>
<td>PANDAR</td>
<td>Up-regulated</td>
<td>Induces proliferation, cell migration and invasion</td>
</tr>
<tr>
<td>PlncRNA1</td>
<td>Up-regulated</td>
<td>Promotes proliferation, cell migration and invasion</td>
</tr>
<tr>
<td>LINC00152</td>
<td>Up-regulated</td>
<td>Promotes proliferation, cell migration and invasion</td>
</tr>
<tr>
<td>THOR</td>
<td>Up-regulated</td>
<td>Induces proliferation, cell migration and invasion</td>
</tr>
<tr>
<td>XIST</td>
<td>Up-regulated</td>
<td>Induces proliferation, cell migration and invasion Targets miR-124</td>
</tr>
<tr>
<td>Rs6871626</td>
<td></td>
<td>Up-regulating IL10 expression</td>
</tr>
<tr>
<td>IncMyoD</td>
<td></td>
<td>Regulation of muscles differentiation</td>
</tr>
<tr>
<td>Inc133b</td>
<td></td>
<td>Regulation of muscles differentiation</td>
</tr>
<tr>
<td>MIAT</td>
<td>Up-regulated</td>
<td>Stimulates proliferation and migration of lens epithelial cells</td>
</tr>
<tr>
<td>TUG1</td>
<td>Up-regulated</td>
<td>Suppresses miR-421, induction of apoptosis</td>
</tr>
<tr>
<td>KCNQ1OT1</td>
<td>Up-regulated</td>
<td>Stimulates proliferation and epithelial-mesenchymal-transition</td>
</tr>
</tbody>
</table>
HOTAIR targets miR-613 and promotes endothelial-mesenchymal transition (EMT) in retinoblastoma cells and its silencing with siRNA resulted in miR-613 upregulation and induced apoptosis (Yang et al., 2018b).

H19 and PANDAR (promoter of CDKN1A antisense DNA damage-activated RNA) are also overexpressed in RB cells. They affect cell proliferation, invasion, and migration through vimentin, CDK1, p53 and E-cadherin regulation (Li et al., 2018a; Sheng et al., 2018).

PlinRNA-1 (prostate cancer-up-regulated long non-coding RNA 1) modulates carbonyl reductase 3 (CBR3) activity, and LINC00152 (long noncoding RNA00152) inactivates Ki-67, Bel-2, and MPM-9 at the post-transcriptional level. In both cases increased proliferation, invasion and migration were observed (Li et al., 2018b; Wang et al., 2018c).

XIST (X-inactive specific transcript) oncogenic activity is associated with its ability to 'sponge' miR-124 and thereby to up-regulate the signal transducer and activator of transcription 3 (STAT3). Its knock-down resulted in significant inhibition of cell proliferation, cell cycle arrest at the G1/G0 phase and the promotion of apoptosis, probably through negative regulation of miR-124/STAT3 axis (Hu et al., 2018a).

Overexpression of THOR (testis-associated highly conserved oncogenic lncRNA) significantly enhances the malignant phenotype transformation of retinoblastoma cells. The process is mediated through up-regulation of c-Myc expression via enhancing its interaction with TGF2BP1 protein (Shang, 2018).

BANCR is also an oncogenic lncRNA, and its overexpression in both retinoblastoma tissues and cell lines is associated with tumour size, choroidal invasion, and optic nerve invasion. Silencing BANCR results in suppression of proliferation, migration, and cell invasion. Its overexpression is correlated with an unfavourable prognosis and, similarly to lncRNAs mentioned above, it can be used as an independent prognostic biomarker for retinoblastoma patients. It is also a promising therapeutic target (Su et al., 2015).

Uveitis

The genetic background of autoimmune diseases is established. Almost 50% of anterior uveitis patients carry the human leucocyte antigen B27 (HLA-B27), Vogt-Koyanagi-Harada (VKH) disease and Behcet’s disease (BD) are both autoimmune syndromes that include uveitis. They are also both associated with several genes, for example, IL17F, IL23A, TNF-AILP3, and HLA-DR4, HLA-DRA53 (VKH) and HLA-B51 (BD). Although many genes are connected with autoimmune uveitis, its exact aetiology is still unclear. Yue et al. revealed that some lncRNAs also are involved in the inflammatory response in VKH and BD patients. Rs6871626 was shown to increase anti-inflammatory cytokine IL-10 production by regulating the LOC285627 gene expression (Yue et al., 2018). Because lncRNAs are relatively recently discovered molecules, their exact role in autoimmune uveitis is yet to be determined.

Strabismus

Strabismus is a common ocular disorder that is caused by the impairment of central neural pathways and maladjusted extraocular muscles (EOMs). EOMs control eye position and play a crucial role in the development of this ailment. Ultrastructural examination of EOMs samples revealed myofilament disintegration, sarcomere destruction, collagen biosynthesis and fibrosis. There are also few lncRNAs regulating muscles differentiation, for example – IncMyed or Inc133h. Ma et al. detected a set of coding and lncRNAs dysregulated in EMOs indicating that they are also involved in the pathogenesis of strabismus, but their participation is not yet fully defined (Ma et al., 2018).

Cataract

Age-related cataract is one of the most common chronic disorders of ageing. Posterior capsule opacification (PCO) is a frequent complication of the cataract surgery. Many morphological and functional changes take place during cataract pathogenesis, including increased proteolysis, altered cell cycle, DNA damage and the change in growth and differentiation of lens epithelial cells (LECs). Shen et al. examined the role of lncRNAs in cataract development. They identified 38 differentially expressed lncRNAs in cataractous lenses, among which MLAT was the most abundant. It was overexpressed in pathological tissue, plasma fraction of the whole blood and aqueous humour of the cataract patients. MLAT was also involved in posterior capsule opacification process, and its knock-down inhibited TNF-2-induced proliferation and migration of LECs. This study provides a novel insight into the pathogenesis of age-related cataract and suggests that MLAT can act as a cataract-specific biomarker and that its silencing can affect PCO formation (Shen et al., 2016; Wan et al., 2017).

Recently a few more lncRNAs were identified to be associated with cataract occurrence. Li et al. observed that TUG1 and caspase-3 were overexpressed and the miR-4211 expression was reduced in cataract lenses compared to healthy tissue. TUG1 negatively regulated miR-421 expression and promoted UV irradiation-induced SRA01/04 cells apoptosis (Li et al., 2017b).

Similarly to TUG1, KCNQ1OT1 is also overexpressed in human cataract lens anterior capsular samples and SRA01/04 cell line treated with H2O2. Down-regulation of KCNQ1OT1 inhibited SRA01/04 cell proliferation and epithelial-mesenchymal-transition (EMT), which are the key phases in cataract formation. This effect was related to the impact of KCNQ1OT1 on SMAD4, a critical intracellular mediator of proliferation and EMT.

Crystallin gene mutations, especially βB2 crystallin (CRYBB2), are associated with cataract formation. Jia et al. identified 149 up-regulated, and 180 down-regulated lncRNAs in CRYBB2 knock-out induced cataract mice model versus healthy mice. The paper highlighted the need for further research on dysregulated lncRNAs (Jia et al., 2018).

In conclusion, the formation of the cataract is an extremely complex issue associated with several mechanisms and complex networks in which lncRNAs play an important role (Chen et al., 2018).

CONCLUSIONS

The complexity of cellular processes still leaves much to be discovered. However, a better understanding of epigenetic relationships in cellular pathways gives us the possibility to develop effective and specific biomarkers and novel therapeutic strategies in various ophthalmologic diseases. As summarized in Table 1, there are several dysregulated lncRNAs and circRNAs in ophthalmologic disorders, but their exact participation in the development of these types of diseases is still poorly understood, and more research is required.
Acknowledgements

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