

Review

Circular RNAs: Novel Promising Biomarkers in Ocular Diseases

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Received: 2018.09.06; Accepted: 2019.02.08; Published: 2019.03.10

Abstract

Circular RNAs (circRNAs) are a novel class of endogenous non-coding RNAs produced by back-splicing. They are found to be expressed in eukaryotic cells and play certain roles in various cellular functions, including fibrosis, cell proliferation, differentiation, apoptosis and angiogenesis. Dysregulated circRNAs are found in several human disorders including malignancy, vascular, inflammatory as well as nervous diseases. Although, increasing evidence suggests that circRNAs may also contribute in different ocular diseases, the outline of circRNAs in ocular diseases remains obscure. In this review we consider the current state of knowledge regarding the potential role and underlying mechanism of circRNAs in ocular diseases including pterygium, age-related cataract, glaucoma, diabetic retinopathy, retinoblastoma, retinal vascular dysfunction and hyperhomocysteinemia induced ocular diseases, emphasizing that circRNAs could be promising biomarkers for the diagnosis and prognosis evaluation. Future circRNAs-targeted intervention may become a novel therapeutic tool for the treatment of ocular diseases.

Key words: circRNAs, non-coding RNAs, ocular diseases

Introduction

Long non-coding RNAs (lncRNAs) are described as RNAs with a minimum of 200 nucleotides in size and doesn't encode proteins. Currently, more than 28,000 long noncoding RNAs (lncRNAs) loci have been documented in the human genome [1]. These lncRNAs genes are usually situated in genomes either in an independent form or coinciding in compound forms alongside other genes [2]. Besides the linear expression found in most genes, messenger RNA (mRNA) and classical lncRNAs transcripts, cells also express circular RNAs (circRNAs) [3].

CircRNAs are a new group of endogenous non-coding RNAs with covalently sealed continuous loop structures and broadly expressed in eukaryotes, including mammals. They are produced either by

exon skipping mechanism or by linking of the 5' splice position with the 3' splice position via back-splicing [4]. CircRNAs are abundantly expressed in a tissue-specific as well as developmental stage-specific design and play a significant part in various cellular functions, including fibrosis, cell proliferation, differentiation, apoptosis, and angiogenesis [5, 6]. Moreover, these circular molecules can regulate gene expression by functioning as micro RNA (miRNA) sponges, RNA-binding protein repossessing mediators, or regulators of transcription [7, 8]. Abnormally expressed circRNAs were found in numerous human diseases including, malignancy, vascular, inflammatory and nervous diseases [9-11].

CircRNAs show a diverse expression outline in

different eye diseases. The mechanism of circRNAs in the ocular diseases is simply emerging. Accumulative evidence had shown that abnormal expression of circRNAs is very closely related with the development, pathogenesis and progression of various ocular disorders [12-14]. Still, their clinical importance in ocular diseases remains concealed. In this review we will consider the current state of knowledge regarding the expression, regulation and the functional aspect of circRNAs in various ocular diseases (Table 1). In addition, circRNAs might be a potentially biomarker for diagnosis along with prognosis evaluation and proper intervention of circRNAs regulation could be a promising therapeutic target for various ocular diseases.

Pterygium

Pterygium is a benign, wing-shaped, common degenerative ocular surface disorder with a high incidence worldwide. The lesion can significantly interfere with vision when there is an encroachment of hyperplastic conjunctival tissue onto the cornea and sometimes become inflamed, leading to discomfort, redness and cosmetic difficulties [15]. It has been revealed that abnormal cell proliferation, defects in wound healing, angiogenesis and cell transformation were linked to the development and advancement of this disease [16]. However, the precise etiology and pathogenesis of pterygium are still unknown.

Table 1. Selected studies on the relationship between circRNAs and ocular diseases.

Ocular diseases	CircRNA	Expression	Related molecules	Phenotypes affected	References
Pterygium	circ_0085020 (circ-LAPTM4B)	Up-regulated in pterygium samplers.	Bcl-2	Affect viability, proliferation, migration, apoptosis of pterygium and epithelial cells <i>in vitro</i> .	[17]
ARC	circHIPK3	Down-regulated in ARC lens capsules and in all three subtypes of ARC patients.	α -SMA miR-193a CRYAA E-cadherin ZO-1	Affect cell viability, proliferation and apoptosis of primary cultured HLECs <i>in vitro</i> via circHIPK3/miR-193a/CRYAA network.	[20]
Retinal neurodegenerative diseases	cZNF609	Up-regulated in rat retinas and aqueous humor of glaucomatous rat models' eyes.	miR-615, METRN	Affect retinal reactive gliosis, glial cell activation, and RGCs survival in rat models of glaucoma <i>in vivo</i> . Regulate Müller cell function directly and RGCs function indirectly <i>in vitro</i> . Regulate Müller cell through cZNF609/miR-615/METRN network <i>in vitro</i> .	[26]
	cZNRANB1	Up-regulated in glaucomatous rat models' retina and in the aqueous humor from the patients with POAG.	miR-217, RUNX2	Affect retinal reactive gliosis, glial cell activation and RGC survival in rat models of glaucoma <i>in vivo</i> . Regulate Müller cell function directly and RGCs function indirectly <i>in vitro</i> . Regulate Müller cell through cZNF609/miR-615/METRN network <i>in vitro</i> .	[27]
DR	circHIPK3	Up-regulated in diabetic retina and retinal endothelial cells following stressors related to diabetes mellitus.	miR-30a-3p VEGFC, FZD4, WNT2	Affect retinal endothelial cell viability, proliferation, migration, and tube formation in HRVECs <i>in vitro</i> via circHIPK3-miR-30a-3p-VEGFC/ WNT2/FZD4 network. Affect retinal vascular dysfunction, vascular leakage, and inflammation in Diabetes Mellitus-induced mice models <i>in vivo</i> via circHIPK3-miR-30a-3p-VEGFC/WNT2/FZD4 network.	[13] [47]
	Circ_0005015	Up-regulated in the plasma, vitreous sample, and fibrovascular membranes of DR patients.	miR-519d-3p, MMP-2, XIAP, STAT3	Regulate endothelial cell proliferation, migration and tube formation of HRVECs <i>in vitro</i> via circ-0005015-miR519d-3p-MMP-2/STAT3/XIAP network.	[30]
RB	hsa_circ_0001649	Down-regulated in RB samples and cells.	Bcl-2,caspase-3 p-AKT, p-mTOR	Affect cell growth and apoptosis in RB cells <i>in vitro</i> via AKT/mTOR signaling pathway. Affect xenograft growth <i>in vivo</i> .	[34]
Vascular endothelial dysfunction	cZNF609	Up-regulated in retinas of diabetic mice and OIR mice at the neovascularization stage.	miR-615-5p, MEF2A	Affect endothelial cell viability, migration, tube formation and apoptosis in diabetic and OIR mice models <i>in vivo</i> . Regulate capillary degeneration and vascular leakage and retinal inflammation response of endothelial cells <i>in vitro</i> via cZNF609/miR-615-5p/MEF2A network.	[38]
Hyperhomocysteinemia induced ocular diseases	mmu_circRNA_21649	Down-regulated in the eyes of CBS lacking mice models.	Grm1	Mitochondrial dysfunction, inflammation, redox imbalance	[44]
	mmu_circRNA_33761	CBS lacking mice models.	Bbs5		
	mmu_circRNA_008614	Up-regulated in the eyes of CBS lacking mice models.	Mlip		
	mmu_circRNA_29109	CBS lacking mice models.	Sp1		

Abbreviations: ARC, Age-related cataract; DR, Diabetic retinopathy; RB, Retinoblastoma; Bcl-2, B-cell lymphoma-2; α -SMA, α -smooth muscle actin; ZO-1, Zonula occludens-1; *RUNX2*, Runt-related transcription factor 2; FZD4, Frizzled 4 gene; MMP2, matrix metalloproteinase 2; XIAP, X-linked inhibitor of apoptosis protein; STAT3, signal transducer and activator of transcription 3; MEF2A, Myocyte-specific enhancer factor 2A. CRYAA: a gene that encodes α A lens protein; METRN: Meteorin, glial cell differentiation Regulator; WNT2: Wnt family member 2, the WNT gene family consists of structurally related genes which encode secreted signaling proteins; MMP-2: matrix metalloproteinase 2, a member of the matrix metalloproteinase (MMP) gene family; RGC: retinal ganglion cell; POAG: primary open-angle glaucoma; VEGFC: vascular endothelial growth factor-C; OIR: oxygen-induced retinopathy; HRVECs: human retinal vascular endothelial cells; HRVECs: human retinal vascular endothelial cells.

Li et al. suggested that aberrant expression of nearly 669 kinds of circRNAs were discovered in pterygium [17]. Among them, circ_0085020 (circ-LAPTM4B) was considerably up-regulated in pterygium samples, and it was demonstrated by circ_0085020 silencing that circ_0085020 promoted the proliferation as well as the migration of pterygium fibroblasts, and inhibited UV-induced apoptosis of pterygium epithelial cells suggesting that this circRNAs could be a promising biomarker for the management of pterygium [17].

Age-related cataract

Age-related cataract (ARC) is the most cause of vision loss among the aged population [18]. Surgery is the only effective treatment option for ARC [19]. However, the high surgical cost has brought huge financial burden to the society. Accumulating studies had clarified that the circRNAs play a vital role in ocular diseases, while the functions of circRNAs in ARC remains to be discovered.

Liu et al. revealed that down-regulation of circHIPK3 expression was found in all three subtypes of ARC compared with the control [20]. *In vitro* study had discovered that the functions of the human lens epithelial cells (HLECs) were regulated by the circHIPK3/miR-193a/CRYAA network. The down-regulation of circHIPK3 could lead to the overexpression of miR-193a, then operating on CRYAA. CRYAA is a completely novel target gene of miR-193a in HLECs and closely associated with the preservation of lens clarity. While the balance of α -crystallins expression was broken-down via this network, the protective effect of α -crystallins would be destroyed. Additionally, circHIPK3 silencing in ARC cases stimulated the HLECs apoptosis mediated by oxidative stress [20].

In conclusion, the role of circHIPK3 as a functional regulator of HLECs via circHIPK3/miR-193a/CRYAA showed a new targeted method for the prevention as well as treatment for ARC.

Retinal neurodegenerative diseases

Glaucoma is a progressive retinal neurodegenerative disease characterized by the degeneration of retinal ganglion cells. It is a major cause of irreversible visual impairment worldwide. The intraocular pressure (IOP) level plays a vital role in the degeneration of retinal ganglion cells [21]. In recent years, circRNAs have come into sight as possible regulators in some neurodegenerative disorders [13, 22, 23]. Though, the exact molecular mechanism of circRNAs in glaucoma induced retinal neurodegenerative progression is still unclear [24, 25].

Wang et al. suggested that cZNF609 expression

was significantly increased in the glaucoma induced retinal neurodegeneration in rat models [26]. CZNF609 silencing eventually protected retinal ganglion cells from the damage triggered by high IOP level and restrained retinal reactive gliosis by directly regulating Müller cell function. CZNF609 was also found to act as a miR-615 sponge and hinder miR-615 activity, resulting in increased METRN, which partly reversed cZNF609 silencing-mediated inhibitory effects on the cell proliferation of retinal glial cells [26].

In another study, cZNRANB1 expression was also found to be dramatically upregulated in retinal neurodegeneration in glaucoma rat model induced by chamber injection of microbeads [27]. CZNRANB1 knockdown by short hairpin RNA (shRNAs) hindered retinal glial cell activation, gliosis, and restored RGC survival *in vivo*. Additionally, cZNRANB1 knockdown indirectly regulated retinal ganglion cells function by directly regulating the Müller cells' function *in vitro*. cZNRANB1 was proved to act as a miRNA sponge, and regulate Müller cells' function via cZNRANB1/miR-217/RUNX2 signaling [27]. Furthermore, Han et al. detected circRNAs in the postnatal rat retina respectively at day P3, P7, and P12. The changes of these circRNAs species were analyzed and some of them were associated with neuronal apoptosis in the developing nervous system [12].

These results suggested that circRNAs (cZNF609 and CZNRANB1) play an important role in retinal neurodegeneration. CZNF609/miR-615/METRN and CZNRANB1-oriented treatments may serve as a potential therapeutic target for the treatment of retinal neurodegenerative diseases.

Diabetic Retinopathy

Diabetic retinopathy (DR) is the major microvascular complication of uncontrolled diabetes mellitus and also the leading cause of visual impairment and blindness worldwide [28]. Existing worldwide prevalence of DR is almost 30% of the patient with diabetes and around 50% people have sight-threatening DR [29]. Despite various treatment options, most cases with DR don't respond well to existing therapeutic methods. Hence, it is necessary to focus on specific biomarkers for the diagnosis and treatment of diabetic retinopathy.

Circ_0005015 was markedly overexpressed in the plasma fraction, vitreous samples and preretinal fibrovascular membranes (FVMs) of diabetic retinopathy (DR) patients [30]. Circ_0005015 silencing evidently inhibited the spheroid sprouting, migration as well as tube formation of HRVECs [30]. Moreover, circ_0005015 might regulated the function of HRVEC

as a sponge of miR-519d-3p *in vitro*, which was closely associated with cell growth, migration and proliferation via acting on the target matrix metalloproteinase (MMP)-2, STAT3, or XIAP genes [30]. MiR-519d-3p mimic transfection was discovered to decrease the expression level of MMP-2, STAT3, along with XIAP in HRVECs, and further reduced proliferation, migration and tube formation of HRVECs [30]. Further, it was explored that 30 circRNAs were dramatically overexpressed in the serum samples of DR patients compared with the serum samples from the control group [14]. However, *in vivo* and *in vitro* studies are required to explain the mechanism of circRNA-mediated DR development. In conclusion, circRNAs participates in the pathogenesis of DR and thus aid as a potential biomarker for the diagnosis and molecular targets for the treatment of DR.

Retinoblastoma

Retinoblastoma (RB), a malignant intraocular tumor originating from embryonic retinal cells, is a sight and life-threatening disease in children, especially infants. Tumor formation is initiated from the alleles mutation of the retinoblastoma tumor suppressor gene RB1 which is located at 13q14 [31]. AKT, a kind of protein kinase, and its downstream effector mTOR were found in numerous regulated signaling pathways related to cancers [32]. Even though AKT/mTOR was discovered in RB progression, the relationship between AKT and circRNAs is still unclear [33].

Recently, hsa_circ_0001649 was found evidently downregulated in a sample from tumor tissues as well as in most RB cell lines [34]. The expression level of hsa_circ_0001649 was closely linked with the tumor size, clinical stages, pathological type and overall survival [34]. Hsa_circ_0001649 was enhanced in Y79 cells and knocked down in WERI-Rb1 cells to verify the biological roles of hsa_circ_0001649 in RB [34]. The results showed that the low expression level of hsa_circ_0001649 acted as a promotor in the progression of RB by regulating cell growth and cell apoptosis *in vitro*. And *in vivo* studies, the transplanted tumor in up-regulated hsa_circ_0001649 group grew obviously slower and the tumors separated from the nude mice weighed much less as compared to the blank vector group [34]. Still, further target genes regulated by hsa_circ_0001649 are required for further investigation.

Furthermore, hsa_circ_0001649 was found to regulate the cell apoptosis and proliferation by AKT/mTOR signaling pathway [34]. To sum up, hsa_circ_0001649 might be a possibly prognostic biomarker as well as the treatment target for RB.

Retinal vascular dysfunction

Ischemic retinopathies, including retinopathy of prematurity (ROP) and diabetic retinopathy (DR), share many similar pathological characteristics such as blood vessel injury and consequential pathological angiogenesis. Vascular dysfunction is commonly related to endothelial cell dysfunction and abnormal gene regulation [35, 36]. Hence, it is essential to reveal the exact biomarker for retinal vascular dysfunction for the prevention and management of vascular complications. It was reported that circRNAs of SIAE and ZNF280C along with other molecules and pathway plays an important part in the progression of ROP [37]. However, the precise mechanism is obscured.

Liu et al. suggested that cZNF609 was dramatically overexpressed under the high glucose condition and oxygen-induced retinopathy (OIR) both *in vivo* and *in vitro* [38]. CZNF609 silencing considerably reduced capillary degeneration, release of inflammatory factors, decreased retinal vessel loss and suppressed pathological angiogenesis *in vivo*. Alternatively, cZNF609 silencing increased the cell viability, apoptosis, proliferation, migration as well as tube formation, and protected the endothelial cell against oxidative as well as hypoxia stress *in vitro* [38]. Moreover, cZNF609 operated as an endogenous miR-615-5p sponge to sequester inhibiting miR-615-5p action which leads to MEF2A overexpression. MEF2A overexpression could release cZNF609 silencing-mediated outcomes on endothelial cell migration, tube formation, as well as apoptosis [38]. These results suggested that the network consisting of cZNF609, miR-615-5p and MEF2A were connected with the retinal vascular dysfunction.

Hyperhomocysteinemia induced ocular diseases

Hyperhomocysteinemia (HHcy) is a metabolic disorder characterized by increased level of homocysteine in plasma due to the deficiency of Cystathionine- β -synthase (CBS) and less commonly due to deficiencies of enzymes involved in de novo methionine synthesis [39, 40]. Regardless of various vascular diseases including coronary, cerebral and peripheral vascular dysfunction, HHcy can result in various ocular diseases such as retinovascular thromboembolic disease and ectopia lentis along with vascular cognitive impairment [41-43]. However, exact mechanism involved in the development of the ocular diseases is obscured.

Singh et al. revealed that 74 circRNAs showed distinction expression profile, out of which nearly 27% were down-regulated while almost 73% were

up-regulated in the eyes of CBS lacking mice models [44]. They also revealed that HHcy could possibly disrupt the complete metabolism of a cell by altering the methylation of key target genes' regulatory elements and influenced the level of gene products as well as disease phenotype by modulating the "genes-mRNAs-miRNAs-circRNAs-proteins" axis. Furthermore, miRNAs regulated gene expressions by inhibiting mRNA translation and circRNAs and miRNAs interact with each other to regulate miRNA functions suggesting that circRNAs might also be involved in various aspects of the ocular biology [44]. In conclusion, circRNAs might play a role in the development and progression of various eye disorders related with homocysteinemia and could be a potential diagnostic as well as the therapeutic target in homocysteinemia induced ocular diseases.

Conclusion

For decades, circRNAs were mistakenly considered as the transcriptional oddities of only limited biological importance [45] [46]. With the development of technologies, circRNAs becomes an innovative issue, more and more researches related to the significance of circRNAs with human disease have been stated. Increasing evidence suggests that circRNAs, being a novel class of endogenous non-coding RNAs, may play an important role in the pathogenesis as well as disease progression in various ocular disease including pterygium, age-related cataract, glaucoma, diabetic retinopathy, retinoblastoma, retinal vascular dysfunction and homocysteinemia induced ocular diseases. CircRNAs had shown different roles and expression patterns in different eye diseases. Dysregulation of circRNAs has been seemed to link with a wide range of biological processes, such as viability, tube formation, apoptosis, and proliferation. However, aberrantly expressed circRNAs may be driven by the same promoters of linear RNAs, possibility of off target effects in siRNA. And the techniques used to identify and validate circRNAs are largely experimental and approaches remain to be standardized. Further in-depth studies are needed. To sum up, circRNAs may aid as a potential biomarker for diagnosis and a prognostic evaluator in ocular diseases. Additionally, circRNAs-target interventions might be a promising therapy against related ocular diseases.

Competing Interests

The authors have declared that no competing interest exists.

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