• Review Article •

LncRNAs in ocular neovascularizations

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Abstract

• The prevalence of eye diseases worldwide is dramatically increasing and represents a major concern in underdeveloped and developed regions. Ocular diseases, previously associated with a higher depression risk, also impose a substantial economic burden on affected families, thus early detection and/or accurate treatment in order to avoid and prevent blindness should be emphasized. Ocular neovascularization (NV), the leading cause of blindness in a variety of eye diseases, is a pathologic process characterized by the formation, proliferation and infiltration of anomalous, tiny and leaky fragile blood vessels within the eye. Genetics have been suspected to play an important role in the occurrence of eye diseases, with the detection of a numbers of specific gene mutations. Long non-coding RNA (lncRNAs) are novel class of regulatory molecules previously associated with various biological processes and diseases, however the nature of the relation and pathways by which they might contribute to the development of corneal, choroidal and retinal NV have not yet been completely elucidated. In this review, we focus on the regulation and characteristics of lncRNAs, summarize results from ocular NV-related studies and discuss the implication of lncRNAs in ocular NV development.

• KEYWORDS: glaucoma; genetics; long non-coding RNA; neovascularization

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INTRODUCTION

The prevalence of eye diseases worldwide is dramatically increasing and represents a major concern in underdeveloped and developed regions, especially sight threatening diseases. Ocular diseases, previously associated with an higher depression risk, also impose a substantial economic burden on affected families, thus the importance of early detection and/or accurate treatment in order to avoid and prevent blindness should be emphasized[1]. Ocular neovascularization (NV), the leading cause of blindness in a variety of eye diseases, is a pathologic process characterized by the formation, proliferation and infiltration of anomalous, tiny and leaky fragile blood vessels within the eye[2]. Occurrence of eye diseases have been assigned to a number of specific gene mutations such as *EIF1AX*, *SF3B1*, *PLCB4*, *GNA11*, *BAP1* and *GNAQ* for uveal melanoma[3-5], and *RB1* for retinoblastoma[6], however, not all diseases exhibit a mendelian mode of inheritances. The non-coding part of the human genome has recently been recognized to possess a crucial functional importance in physiology and normal development. And this discovery has attracted attention on its potential to contribute to diverse disease etiology[7]. Long non-coding RNA (lncRNAs) are transcripts possessing a size within 200 to 100 000 nucleotides, structurally resembling mRNA and presenting little to no protein-coding potential and can be classified into several types according to their genonic locations. Although most lncRNAs are located in the nucleus[8], however nearly 15% can be found present in the cytoplasm[9]. LncRNAs can be classified as sense or antisense, the former comprising of those that overlap with proteing-coding genes. If the promoter and transcript are situated in proximity, the lncRNA is then said to be bidirectional[10-11].

Many studies reported lncRNAs participation in numerous biological processes, including stem cell maintenance, and cellular phenotype differentiation[12]. Transcriptinal regulation may be influenced by LncRNAs *via* several modes such as decoy, signal, guide and scaffold[13]. They might also as signals in response to multiple stimuli, participate in recruiting corresponding complexes in order to directly or indirectly silence or activate the expression of a gene[14]. In addition, some lncRNAs may influence the expression of genes through post-transcriptional events, and also participate in the modification process post translation[15]. Nonetheless, lncRNAs have been previously associated with neurodegenerative diseases[16], multiple tumors and cancers[17-18], and common ocular diseases such glaucoma[19], and diabetic retinopathy[20], among others, however the nature of the relation and pathways by which they might contribute to the development of corneal, choroidal and retinal NV have not yet been completely elucidated.

In this review, we review pathophysiology and risk factors for developing ocular NVs focus on the regulation and characteristics of lncRNAs, summarize results from ocular NV-related studies and discuss the implication of lncRNAs in ocular NV development

LNCRNAS IN OCULAR NEOVASCULARIZATIONS

Corneal Neovascularization

Definition Corneal diseases are considered one of the main causes of irreversible blindness, and corneal neovascularization (CN) can be observed in the majority of affected cases. Further investigations have shown that angiogenesis negatively impacts the prognosis for individuals undergoing keratoplasty procedures[2].

Normal corneal transparency is an essential factor in providing appropriate anterior refractive surface. Corneal transparency and optimal vision require an important aspect of corneal pathophysiology which is the avascularity of the corneal stroma. Decrease in corneal transparency associated with the development of NV. The presence of vascularization, is mostly linked to pathologic conditions. And may cause visual acuity deterioration[21]. Characterized by the development and invasion of neovessels-from the limbus to the cornea, CN mainly results from oxygen deprivation provoked by disequilibrium between corneal transparency preserving factors and lead to proliferation and migration of vascular endothelial cells into the corneal stroma. The newly formed vessels may then cause inflammation, oedema, protein and lipid deposition, therefore posing a considerable threat to both visual acuity and corneal transparency and, contribute to corneal grafts rejection[22-24]. It is currently estimated that approximately 1.4 million new cases of CN are registered every year, 12% of whom eventually leading to visual loss[25].

Pathogenesis The upregulation of angiogenic cytokines mediates the in-growth of new blood vessels. Cornea's basement membrane and extracellular matrix are degraded by the metalloproteinase enzyme, while the vascular epithelial cells invasion into corneal's stromal layer is facilitated by proteolytic enzymes. Inflammatory cells produce angiogenic factors such as VEGF and fibroblast growth factors during inflammation process. With the former regulating the production of matrix metalloproteinases by endothelial cells, thus paving the way for the formation of new blood vessel[26].

Risk factors CN can be caused by congenital diseases, autoimmune conditions, inflammatory disorders, infections, hypoxia, chemical burn, limbal stem cell deficiency, corneal graft rejection, and traumas. CN within the cornea usually results from ulcers, trachoma, different types of keratitis and conjunctivitis. Superficial presentations of CN usually results from hypoxia caused by contact lenses, whereas chronic inflammatory and anterior segment ocular diseases are considered main causes for deep presentations of CN[27].

LncRNAs in corneal neovascularization

LncRNA NR_033585 and lincRNA chr8:129102060–129109035 A recent study[28] tried to highlight the possible role played by lncRNAs in the development of CN, and consisted in using alkaline solution to stimulate CN development in C57BL/6 mice eyes and lncRNA expression profiling in order to compare expression differences in lnRNA among normal and vascularized corneas. The observation of approximately 154 differentialy expressed lncRNAs then prompted researchers to randomly selected a number of 6 lncRNAs (3 up-regulated and 3 down-regulated) and proceed to a comparison between their expression patterns and antiangiogenic factors and proangiogenic factors[29]. The result showed similar expression pattern between proangiogenic factors, such as Ang-2, MMP-9 and VEGF, and up-regulated lncRNAs (lncRNA NR_033585), whereas down-regulated lncRNAs (lincRNA chr8:129102060-129109035) did exhibit expression patterns that were similar to antiangiogenic factors such as platelet derived growth factor (PDGF)[30].

Further investigations conducted with the aim to explore the possible correlation existing between abberant lncRNAs expression and CN development suggest that lncRNA NR_033585 may potentially act as an proangiogenic factor, whereas the reverse strand of lincRNA: chr8:129102060-129109035 might play an antiangiogenic role during the development process of CN[31].

Choroidal Neovascularization

Definition Choroid is located between the retina and the sclera. Its main function consists of supplying nourishment and moistening the volume and temperature of the ocular globe. Despite the fact that the choroidal circulation is a relatively low oxygen content, it still accounts for nearly 85% of the total blood flow in the eye[32].

choroidal neovascularization (CNV), a pathologic condition whose main characteristics are abnormal growth of new blood vessels in the choroid, and if left untreated, it can cause a rapid deterioration in central vision and color recognition especially when the subfoveal region is being affected[33]. CNV is considered a main cause of central visual loss in both adults[34] and children[35-36]; despite possessing a lower blindness prevalence, however children, because of a higher disability-adjusted life years (DALY)[37] have been shown to face greater challenges not only in education but also in emotional development[38-39].

New blood vessels, formed from the choroid, penetrate into the subretinal pigment epithelial and subretinal space through the Bruch membrane. Bleeding and leaking from these newly formed vessels can lead to hemorrhage and to exudative retinal

detachment[40], and may cause destruction of photoreceptors and vision loss (blindness). Although CNV is considered to be a multifactorial lesion and its development is known to be induced by numerous stimuli, the involvement of macrophages and VEGF and alterations in Bruch's membrane, take the main role for the development of this disease[41].

Pathogenesis Vascularization in the choroid develop when newly formed blood vessels start to grow into the sub-retinal space through a break in the Bruch membrane. The presence of abnormaly elevated VEGF levels, and its counteraction by retinal epithelia produced protein, also called pigment epithelium derived factor (PEDF) are the main inducer of new blood vessels growth. Maintening the balance between VEGF and PEDF is thought to be one of the main determining factor in the development or progression of CNV[42-43].

Risk factors CNV can be observed in various ocular diseases including adult macular degeneration (AMD)[44], pathologic myopia (PM)[45] and ocular histoplasmosis syndrome (OHS)[46]. However, choroid's primary functions can be affected by any condition capable of disrupting the Bruch's membrane, thus increasing the risk of developing CNV. The incidence and progression of AMD are related to age and genetic factors[47]. The activity of lysosomes for external segments degradation of photoreceptors decreases proportionally with age, ultimately leading to an lipofuscin accumulation, thus affecting normal function of retinal pigment epithelium (RPE)[48]. Furthermore, soft drusen represent another important risk factor for CNV development[49].

Several other risk factors have been previously been reported, including age[50], smoking[51], hyperglycemia[52], dietary intake of omega-3 fatty acids[53] and vegetables and fruit with antioxidants including lutein[54] and zeaxanthin[55], as well as, hereditary or traumatic or inflammatory disorders[56].

LncRNAs in choroidal neovascularization

Vax2os1 and Vax2os2 A recent research tried to investigate the possible changes in VEGF and lncRNAs expression *via* the exposition of mice to hyperoxic condition for a period of 5d in order to induce ocular NV; whereas age-matched control groups were kept in room air. The result showed disappearance of existing capillaries after exposure to 75%±2% oxygen, even though peripheral retina remained vascularized. Microarray data demonstrated that lncRNAs expression level were different among case and control group[57].

Furthermore, aqueous humor samples were collected from patients affected with exudative AMD (case, n=10) and those with cataract surgery (control, n=10) and later submitted to microarray analysis and revealed a significant up-regulation in Vax2os1,Vax2os2 and VEGF expression, among patients presenting ocular NV, thus suggesting their possible importance as potential biomarkers[58].

Vax2os1 and Vax2os2, are antisense transcripts of the Vax2 gene, highly expressed in the choroid and retinal vasculature. A strong RNA-protein interactions existing not only between Vax2os1 and C1D, but also between Vax20s2 and PATL2, might play a undeniably important part in CNV pathogeneisis because C1D and PATL2 serve as important regulators of chromatin structure stability[59-60]. Increased information about these two lncRNAs will facilitate a greater understanding of CNV pathogenesis. Provided that each lncRNA regulates specific facets of protein activity, a more refined and less toxic drug targeting a lncRNA may be employed for CNV treatment.

Retinal Neovascularization

Definition Retinal NV refers to the formation of neovessels originating from and contiguous with the pre-existing retinal vascular bed and generally occurs in ischemic retinopathies in which the damage to retinal vessels results in retinal ischemia.

Pathogenesis The newly formed blood vessels are located either within or near the retina, in portions where blood vessels are not usually present. Such new vessels are actually generated from the blood vessels in the retina and the NV may remain within the plane of the inner retina, grow inwards toward the vitreal surface and escape from the internal limiting membrane, or extend downward to the inner nuclear layer and go as far as the RPE.

Risk factors The most prevalent is risk factors for retinal NV are: diabetic retinopathy, retinal vein occlusions[61]. Other risk factors include Behcet's disease[62], sickle cell hemoglobinopathy[63], sarcoidosis[64], Eales disease[65], and systemic lupus erythematosus[66].

LncRnas in retinal neovascularizations Myocardial infarction-associated transcript (MIAT) has been previously associated with myocardial infarction ,is reportedly highly expressed in retinal precursor cells[67].

Yan *et al*[68] tried highlighted its involvement in microvascular dysfunction caused by diabetes mellitus through quantitative polymerase chain reaction (PCR) to evaluated its expression in both diabetic retinas and endothelial cells placed in high glucose medium. Their research not only revealed an up-regulation of MIAT level post high-glucose or oxidative stress treatment and also a proliferation and migration of endothelial cell ultimately causing microvascular dysfunction this up-regulation contributes to endothelial cell proliferation and migration, thus leading to microvascular dysfunction. Further investigation on therapeutic effects of prompted them to perform an MIAT down-regulation which led to inhibition of endothelial inflammatory responses. This might be due to the fact that MIAT might act as competing endogenous RNA in VEGF regulation and thus participate in the development of retinal NV. A recent hypothesis also states that by competing specifically for shared miRNAs, RNA transcripts sharing

miRNA-binding might be able to communicate and regulate each other[69]. Which could explain how MIAT by binding to the same site as miR-150-5p, might alleviate repression effect of miR-150-5p and cause up-regulation of its target gene VEGF. Additionally, inhibition of upregulation of both TNF-α and ICAM-1 can be provoked by MIAT knockdown,thus reducing inflammaton and vascular leakeage[70].

MALAT1 is a long non-coding RNA highly expressed in the nucleus, and located on chromosome 11q13[71] in human, and 19qA[72] in mouse. It is one of the most studied lncRNAs and has been identified in a wide range of tumors, including lung cancer, liver cancer, renal cell carcinoma, bladder cancer, and osteosarcoma[73], and microvascular dysfunction caused by diabetes[74]. The expression level of *MALAT1* has been shown to be similar to protein-coding genes such as β-actin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH)[75], and this findng is the main catalyst for the conduction of large amount of researches in order to provide underlyning insights of its functions.

Gene epression can be regulated by *MALAT1*, which has also been showed to play a positive role in tumor cell proliferation, invasion, migration and apoptosis[76]. Recent studies not only showed that the cerebellum, hippocampus and brain stem cells of alcoholics, and tumors affected patents harbor up-regulated level of *MALAT1*, which hints at its possible role in neurodegeneration[77], but also revealed that hypoxia, hyperglycemia and oxygen deprivation are the main causes of its up-regulation[78].

Furthermore, a genetic *MALAT1* deletion in mice resulted in a reduction of retinal vascular growth and the presentation of an endothelial proliferation. Furthermore, both cultured mouse primary skeletal muscle microvascular endothelial cells followed by 24h reperfusion and mouse gastrocnemius muscle that underwent hindlimb ischemia followed by 28d of reperfusion were shown to harbor increased level of both *MALAT1* and *VEGFR2*. *MALAT1* silencing through a locked nucleic acid (LNA)-GapmeRs method, leads to a reduction in tube formation, cell proliferation and migration, suggesting an important role played by *MALAT1* in angiogenesis through direct regulation of VEGFR2 and that genetic *MALAT1* deficiency could provoke an angiogenesis reduction and perfusion[79].

Yan *et al*[80] were among the first to perform lncRNA expression profiling of mouse retinas with the aim to identify the possible lncRNAs implication in early diabetic retinopathy, followed by the conduction of a real-time PCR for the detection and comparison of expression pattern between clinical samples and RF/6A cell model of hyperglycemia. The results showed aberrant expression in approximately 303 lncRNAs (comprising of 214 down-regulated and 89 up-regulated), in early diabetic retinopathy. Additionally, MALAT1, was not only found to be up-regulated in the mouse model of hpyperglycema, but also in both the aqueous humor and fibrovascular membranes of diabetes-affected patients. LncRNAs by participating in the modulation of numerous pathogenic pathways contribute to the pathogenesis of diabetic retinopathy. MALAT1 could possibly serve as a therapeutic target for the prognosis, diagnosis and treatment of diabetic retinopathy[22].

Liu *et al*[81] emphasized the role played by MALAT1 in retinal vasculature remodeling by demonstrating its upregulated expression in both STZ-induced diabetic rats and db/db mice retinas. Furthermore, the crosstalk between MALAT1 and p38 MAPK signaling pathway being involved in the regulation of endothelial cell function, an MALAT1 knockdown could alleviate *in vivo* microvascular dysfunction and through affecting phosphorylated p38 MAPKs levels provokes inhibition of tube formation, endothelial cell proliferation, ultimately ameliorating prognosis of diabetic retinopathy[79].

Michalik *et al*[82] focused their researches on expression characterization of MALAT1 in human endothelial cells). The study showed an upregulation of MALAT1 level post hypoxia induction, and also confirmed that the endothelial cells proliferation and neonatal retinal vascularization could be respectively inhibited and reduced after an MALAT1 genetic ablation, whereas a pharmacological MALAT1 inhibition causes the reduction of blood flow recovery and capillary density.

CONCLUSION

Ocular NV, the leading cause of blindness in a variety of eye diseases, is a pathologic process characterized by the formation of anomalous blood vessels within the eye. LncRNAs are novel class of regulatory molecules previously associated with various biological processes and c diseases, however the nature of the relation and pathways by which they might contribute to the development of corneal, choroidal and retinal NV have not yet been completely elucidated.

In summary, we discussed potential implications of lncRNAs such as Vax2os1, Vax2os2, lncRNA NR_033585, lincRNA chr8:129102060-129109035, MIAT, and MALAT1 in the development of ocular NVs. Nevertheless, more in depth research are needed to identify large number of ocular NV-associated lncRNAs and elucidate their involvement in the development of this condition. We stress that this article creates a paradigm for future studies of lncRNAs in the prevention, diagnosis and might provide therapeutic targets for treatments, and help avoid vison loss and vsual impairment caused by ocular NV.

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