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Current Genomics, 2022, *23*, 246-261

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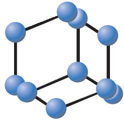

Long Non-coding RNAs: Pivotal Epigenetic Regulators in Diabetic Retinopathy

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ARTICLE HISTORY

Received: April 04, 2022
Revised: May 07, 2022
Accepted: May 16, 2022

DOI:
10.2174/1389202923666220531105035

Abstract: Diabetic retinopathy (DR) is a severe complication of diabetes; however, its mechanism is not fully understood. Evidence has recently revealed that long non-coding RNAs (lncRNAs) are abnormally expressed in DR, and lncRNAs may function as pivotal regulators. LncRNAs are able to modulate gene expression at the epigenetic level by acting as scaffolds of histone modification complexes and sponges of binding with microRNAs (miRNAs). LncRNAs are believed to be important epigenetic regulators, which may become beneficial in the diagnosis and therapy of DR. However, the mechanisms of lncRNAs in DR are still unclear. In this review, we summarize the possible functions and mechanisms of lncRNAs in epigenetic regulation to target genes in the progression of DR.

Keywords: Diabetic retinopathy, lncRNAs, miRNAs, epigenetics, regulator, histone modification.

1. INTRODUCTION

Diabetic retinopathy (DR), the most common microvascular complication of diabetes mellitus (DM), is occurring at increasing rates across the globe [1]. Most patients who experience DR will ultimately develop vision loss, which seriously threatens health. DR is characterized by angiogenesis, inflammation, and apoptosis, but the pathogenesis of DR is not very clear, and the treatments for DR, including laser treatments, vitreoretinal surgery, and application of anti-vascular endothelial growth factor (VEGF) agents, are not always effective [2, 3]. Therefore, it is significant to identify novel biomarkers for the treatment of DR [4].

Growing evidence has shown that long non-coding RNAs (lncRNAs) may play regulatory roles in physiological processes and various diseases, including DR [5-9]. A great number of lncRNAs are dysregulated during DR across multiple pathways involved in angiogenesis, apoptosis, and inflammation [5, 10-13], such as imprinted maternally expressed transcript (*H19*), maternally expressed gene 3 (*MEG3*), HOX antisense intergenic RNA (*HOTAIR*), myocardial infarction associated transcript (*MITA*), metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*), and more [14-19]. However, the detailed mechanisms involved in the occurrence and development of DR have not been fully defined [5]. The study of the possible involvement of lncRNAs in DR is important and may identify novel biomarkers for the diagnosis and treatment of DR. In this article, we review the regulatory roles of lncRNAs and the possible mechanisms in DR.

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2. CLASSIFICATION OF LncRNAs

LncRNAs belong to non-protein-coding RNAs (ncRNAs), which include two major groups: structural non-coding RNAs and regulatory non-coding RNAs. Regulatory non-coding RNAs can generally be classified into small, medium, and long non-coding RNAs according to the length of their nucleotides [20-22]. Among these, lncRNAs are longer than 200 nucleotides, and more than half vary from 1000 to 10,000 bases [23].

Most lncRNAs can act as epigenetic regulators to regulate gene expression involved in biological processes [24, 25]. All lncRNAs are transcribed in the nucleus, and approximately 54% are exported to the cytoplasm [26, 27]. Thus, lncRNAs play regulatory roles both in the nucleus and cytoplasm, and the various roles of lncRNAs depend on the different locations [28]. Nuclear lncRNAs can bind DNA and protein to regulate gene expression by connecting to the nuclear matrix and modifying the structure of chromatin [29-31]; cytoplasmic lncRNAs can regulate mRNAs by acting as microRNAs (miRNAs) sponges [28].

In this review, we focus on the functions that some lncRNAs play as scaffolds in the nucleus and sponges in the cytoplasm in DR. Individual examples will demonstrate the roles of lncRNA, contributing to improvements in clinic diagnoses and treatment in DR.

3. LncRNA ACTS AS SCAFFOLDS FOR HISTONE MODIFICATION COMPLEXES TO REGULATE TARGET GENES

The state of chromatin and the remodeling of chromatin are crucial for gene transcription in epigenetic regulation. Chromatin remodeling involves both nucleosomal structure and histone modifications. Recent research has revealed that

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Current Genomics

epigenetic regulation is also pivotal in the pathogenesis of DR. Some lncRNAs, such as *HOTAIR*, *MALAT1*, and antisense non-coding RNA in the INK4 locus (*ANRIL*), are able to regulate gene expression by remodeling the state of chromatin [10, 18, 19, 32]. Clarifying the epigenetic mechanisms between lncRNAs and DR will help us learn more about the pathogenesis of DR.

3.1. LncRNA-*HOTAIR*

HOTAIR is ~2000 bp in length, and it is transcribed from the Homeobox C (HOXC) locus [33]. *HOTAIR* is essential for regulating cell proliferation, cell cycle, and apoptosis. It is reportedly deregulated in many cancers [33, 34]. Moreover, it may act as a scaffold of the complex of histone modification [32, 33, 35-37] and can regulate the transcriptional activity of target genes by binding to two gene expression repressing complexes, polycomb repressive complex 2 (PRC2), a histone methylase, and lysine-specific demethylase 1 (LSD1), a histone demethylase [32, 33, 35, 38]. PRC2 consists of embryonic ectoderm development (EED), suppressor of zeste 12 homolog (SUZ12), and enhancer of zeste 2 (EZH2). As a histone methylase, PRC2 is mainly in charge of the methylation of H3 Lys 27 (H3K27), whereas the histone demethylase LSD1 is able to catalyze H3K4 or H3K9 histone demethylation [35, 39]. By remodeling the states of histone and chromatin, *HOTAIR* functions crucially in carcinogenesis and metastasis [32, 33, 35-39].

HOTAIR has also been proven to be abnormally expressed in DR. It was found to be up-regulated in the serum of patients with DR, the retina of DM model mice, and the retinal endothelial cells (mREC) of mice under high glucose (HG) treatment. Thus, HOTAIR is considered a marker of DR diagnosis and prognosis [18, 40]. Zhao *et al.* found that *HOTAIR* knockdown inhibited the proliferation of mRECs under HG conditions and inhibited cell invasion, migration, and hyperpermeability induced by HG treatment. In addition, *HOTAIR* knockdown was reported to relieve the retinal vessel impairment of mice with DM. Furthermore, *in vitro* and *in vivo* experiments demonstrated that *HOTAIR* knockdown significantly increased vascular endothelial-cadherin (VE-cadherin) expression but decreased VEGF expression [18]. Both VE-cadherin and VEGF are important in the progression of DR [41, 42] as major regulators: the higher expression level of VEGF promotes angiogenesis, and the lower expression level of VE-cadherin can accelerate the leakage of the blood-retinal barrier (BRB) [43, 44]. It is essential to understand more about the mechanisms that regulate VE-cadherin and VEGF. Zhao proved that knockdown of HOTAIR improved the function of mRECs under HG treatment *in vitro* and inhibited the damage of retinal vessels *in vivo* by decreasing the expression of VEGF and increasing the expression of VE-cadherin [18]. The effects of HOTAIR knockdown suggest that HOTAIR is a potential therapeutic target for DR.

Zhao *et al.* found that the knockdown of *HOTAIR* only altered the H3K4me3 level on the VE-cadherin promoter but did not affect the H3K9me3 and H3K27me3 levels. Hence, demethylation of H3K4me3 was induced by LSD1 binding to *HOTAIR,* resulting in the inhibition of VE-cadherin expression (Fig. **1A**) [18]. *HOTAIR* could enhance the binding of LSD1 to the VE-cadherin promoter and reduce the H3K4me3 level, which, in turn, suppressed the transcriptional expression of VE-cadherin. Conversely, the silencing of *HOTAIR* reversed this effect [18].

Zhao *et al.* also explored the effect of *HOTAIR* on the histone methylation of the VEGFA promoter and found no effects on the H3K4me3, H3K27me3, and H3K9me3 levels, suggesting that histone methylation did not play a regulatory role in the expression of VEGFA [18]. *HOTAIR* might affect VEGFA transcription by other mechanisms, which are different from those of VE-cadherin. For example, many studies have reported that hypoxia-inducible factor 1α (HIF1α) can act as a transcription factor to promote the expression of VEGFA [45, 46]. HIF1α may bind to LSD1 and *HOTAIR* and form a complex for binding the VEGFA promoter, thereby regulating the VEGFA promoter activity (Fig. **1B**). *HOTAIR*, acting as a scaffold of LSD1, regulates expressions of VEGFA and VE-cadherin *via* LSD1 and HIF1α, respectively. As a result, *HOTAIR* may lead to microvascular dysfunction and aggravate the progression of DR.

HOTAIR recruits both PRC2 and LSD1 to affect the state of chromatin and plays a key role in carcinogenesis [32, 33, 35]. For example, *HOTAIR* can repress gene expression by increasing H3K27me3 and decreasing H3K4me3 in lung cancer [47]. Knockdown of *HOTAIR* altered the H3K4me3 level but not the H3K27me3 level on the VE-cadherin promoter under HG conditions [18]. The detailed mechanism for *HOTAIR* binding to PRC2 in the progression of DR is still unclear. When we consider the biomarker function of *HOTAIR*, there is no doubt that *HOTAIR* may be an attractive target in diagnosis and therapy. Hence, additional research is urgently needed.

3.2. LncRNA-*MALAT1*

MALAT1, an intergenic lncRNA located on human chromosome11q13.1, was first discovered in non-small cell lung carcinoma [48]. *MALAT1* is involved in various diseases, such as solid tumors, heart disease, diabetes, and DR [5, 48-51]. *MALAT1* is significantly up-regulated in DM and DR [5, 19, 51, 52]. *MALAT1* dysregulation is a potential molecular mechanism of DR pathogenesis, but the underlying mechanism remains unclear.

MALAT1 may induce retinal microvascular dysfunction and increase glucose-induced inflammatory response [49, 52]. *MALAT1* expression was higher in the vitreous humor of patients with diabetes and human retinal endothelial cells (HRECs) exposed to HG and inflammatory markers (interleukin-6 [IL-6], tumor necrosis factor-alpha [TNF-α], monocyte chemotactic protein-1[MCP-1] and IL-1β) were also increased. Furthermore, when *MALAT1* was silenced by a siRNA-mediated knockdown approach, all the markers of inflammation, including the components of PRC2 (EZH2, EED, and SUZ12), decreased. Thus, *MALAT1* is believed to act as a scaffold to chromatin-associated complexes to regulate the expressions of genes that recruit PRC2 in DR [19, 53]. *MALAT1* might also increase H3K27me3 levels by enhancing the targeting of EZH2 [54]. Upregulation of *MALAT1* may enroll PRC2 to repress anti-inflammatory genes that are increased in DR (Fig. **2**). Biswas *et al.* developed a murine model in which *MALAT1* was knocked out (M1 KO)

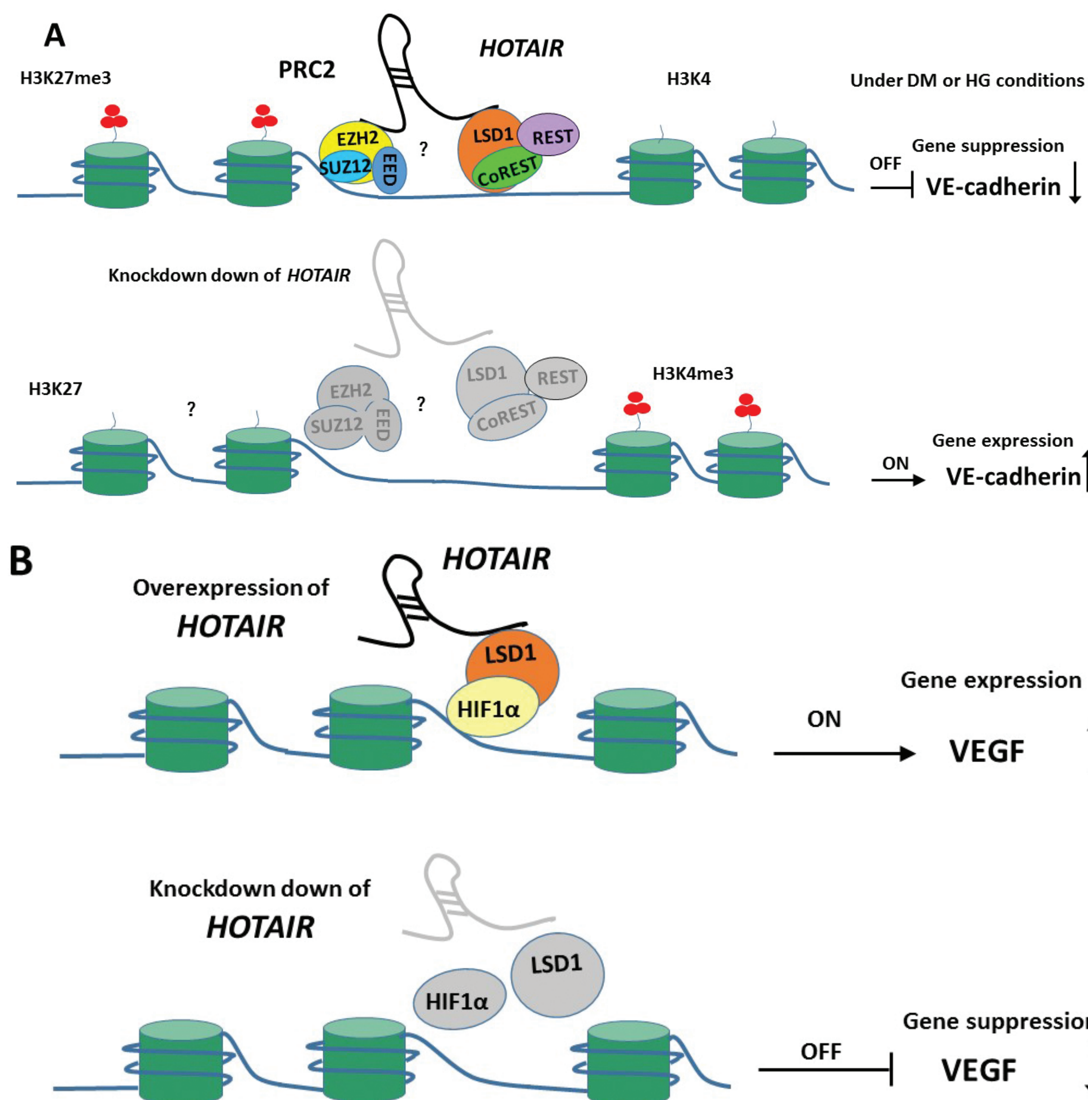

Fig. (1). *HOTAIR* regulates target gene expression as scaffolds for histone modification complexes. (**A**) *HOTAIR*/LSD1 inhibits the transcriptional expression of VE-cadherin *via* reducing the H3K4me3 level on its promoter in diabetic retinopathy. The overexpression of *HOTAIR* enhanced the binding capacity of LSD1 to the VE-cadherin promoter and reduced the H3K4me3 level to suppress the transcriptional expression of VE-cadherin. The silence of *HOTAIR* reversed this effect. Knockdown of *HOTAIR* repressed the binding capacity of LSD1 to the VE-cadherin promoter and increased the H3K4me3 level to activate the transcriptional expression of VE-cadherin. (**B**) *HOTAIR*/LSD1 and HIF1α form a complex to bind VEGF promotor and activate the transcription of VEGFA in diabetic retinopathy. *HOTAIR* binds to LSD1 and HIF1α, forming a complex for binding the VEGFA promoter, thereby activating the expression transcription of VEGFA. The knockdown of *HOTAIR* suppresses the binding of LSD1 and HIF1α to the VEGFA promoter, thereby suppressing the expression transcription of VEGFA. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

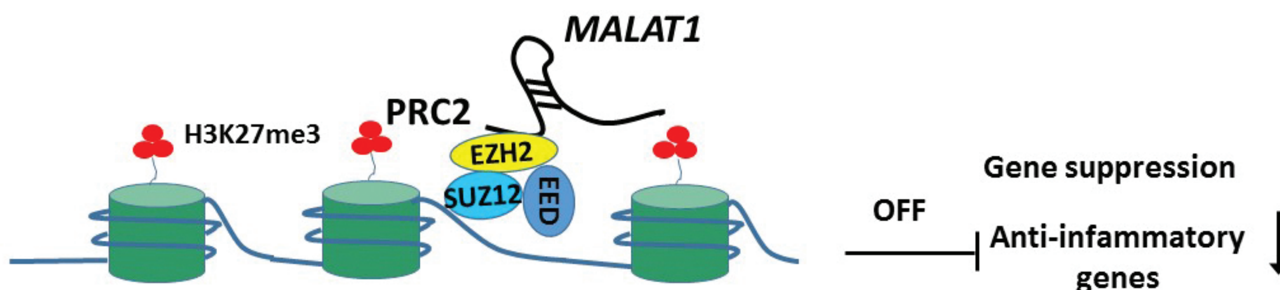

Fig. (2). *MALAT1* regulates anti-inflammatory activity through independent pathways (an epigenetic mechanism) in diabetic retinopathy: *MALAT1* recruits PRC2 (EZH2, EED, and SUZ12) to augment H3K27me3 levels and repress transcription of anti-inflammatory genes. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

to confirm the possibility that *MALAT1* could provoke inflammation. They observed that when *MALAT1* transcripts were depleted in the retinal tissues of M1 KO mice, all inflammatory markers and components of PRC2 transcripts were significantly downregulated, and the diabetic phenotype was prevented. Thus, expression of *MALAT1* is necessary for inflammation. It was found that regulation of *MALAT1* is associated with epigenetic modification. The expression of *MALAT1* and components of PRC2 decreased in the presence of DZNep, an inhibitor of histone methyltransferases. Similar results were obtained for the expression of TNF-α but not for the expression of IL-1β, IL-6, and MCP-1. Surprisingly, expressions of these cytokines were found to be up-regulated. However, results differed in the presence of DNA methyltransferase inhibitors. The expressions of all the cytokines, including inflammatory factors IL-1β, IL-6, MCP-1, and *MALAT1,* increased.

The diversified expression data can perhaps be explained by the various regulating roles of *MALAT1*, which are based on different epigenetic mediators (*e.g*., different methylation states for DNA or histones). Biswas *et al.* proposed two different epigenetic mechanisms for *MALAT1* in the regulation of inflammatory processes in DR, one through an independent pathway and the other through a dependent pathway [19]. They suggested that DNA methylation plays an important role in regulating the transcription of *MALAT1* in the independent pathways. The upregulation of *MALAT1* may enroll PRC2 to repress anti-inflammatory genes, which might subsequently increase the transcription of inflammatory genes. Conversely, it was proposed that the *MALAT1* gene might affect inflammatory genes directly to promote an inflammatory response in DR *via* a dependent pathway [19]. As *MALAT1* knockdown could decrease inflammation and diminish diabetic damage, depletion of *MALAT1* is a potential approach for DR treatment. However, so much is unknown in this field. Additional exploration must uncover the mechanism of how *MALAT1* impacts inflammation in DR.

3.3. LncRNA-*ANRIL*

After antisense non-coding RNA in the INK4 locus (*ANRIL*) was first identified in melanoma neural tumor syndrome [55], it was associated with many kinds of cancers [34, 56, 57]. *ANRIL* also appears to be a genetic maker for DR [10]. Compared to healthy control groups, *ANRIL* levels were found to be higher in body fluids in patients with DR and in retinal tissues of rats with DR [58, 59]. *ANRIL* expression is closely related to the progress of DR as well; the higher the expression, the more severe the symptoms of DR. Upregulation of *ANRIL* can activate the nuclear factor kappa-B (NF-κB) pathway as well as other related factors, including angiotensin II (Ang II), AT1R, p65, and VEGF [58, 59].

Thomas *et al.* revealed that the expressions of *ANRIL* and VEGF can be up-regulated in HRECs exposed to HG treatment and in animal models of diabetes [10]. They measured the expression of EZH2 and found a significant downregulation of EZH2 levels in *ANRIL* knockout (KO) mice. In the presence of DZNep, all components of the PRC2 complex were suppressed, as were mRNA expression levels for both VEGF and *ANRIL*. These results suggested a regulatory effect of PRC2 on *ANRIL* and VEGF.

Thomas *et al.* have proven that VEGF is also regulated by miR-200b through p300 [10, 60]. *ANRIL* may work within the PRC2 complex and may have a regulatory role in p300 [10, 61, 62]. Furthermore, interactions among *ANRIL*, EZH2, and p300 were explored by RNA immunoprecipitation (RIP) assay. Data showed that the binding of *ANRIL* to p300 and EZH2 increased significantly in HG conditions, which strongly indicated that *ANRIL* was directly bound to both p300 and EZH2 to regulate the expression of VEGF under HG conditions. Conversely, the expression of VEGF was reduced along with the level of EZH2 when *ANRIL* was silenced in transfected *ANRIL* siRNA in the HRECs and *ANRIL* KO murine models. *ANRIL* might participate in the pathogenesis of DR by indirectly regulating VEGF [10].

VEGF is the core factor for the development of DR, especially through angiogenesis. Abnormally high expression of VEGF can induce BRB permeability and enhance cell migration and tube formation, all of which are critical for stimulating the angiogenic process in DR. Decreasing the expression of VEGF using an anti-VEGF therapy is a key therapeutic approach for DR. As *ANRIL* positively regulates the expression of VEGF, the expressions of both *ANRIL* and VEGF is increased aberrantly in DR. Additional research using antagonists to silence the *ANRIL* to inhibit VEGF is necessary to learn more about treatments for DR.

Wei *et al.* demonstrated that *ANRIL* was upregulated sharply in rats with DR [59]; however, apoptosis and inflammation triggered by HG conditions were retarded in the retinal tissue of *ANRIL* knocked-down rats with DR. The vascular function in *ANRIL* knocked-down rats with DR could be ameliorated. Furthermore, the knockdown of *ANRIL* suppressed the NF-κB pathway. All the research work by Thomas *et al.* and Wei *et al.* suggests that *ANRIL* may be significant for the development of DR, so silencing *ANRIL* would be useful in DR treatments [10, 59].

LncRNAs not only regulate the expression of proteins by epigenetic modification but also modulate the transcription of RNA, such as micro-RNA (miRNA). MiRNAs, a class of short non-coding RNA, are 18 to 24 nucleotides long. They can directly bind to the 3' domain of their target mRNAs to silence the translation of a target gene [21, 63, 64]. In recent years, miRNAs, including lncRNAs harboring miRNA, have been associated with the occurrence and development of DR [11, 65]. In fact, that lncRNA binding miRNA is an important post-transcriptional regulation mechanism, which is believed to be a miRNA sponge to compete with target mRNA to regulate gene expression [13, 66]. These lncRNAs are known as competitive endogenous RNA (ceRNA). LncRNA-miRNA-mRNA networks (or ceRNA networks) are significantly related to DR [5, 12, 13, 67]. The mechanisms have been investigated. In the following sections, some examples are provided to explain how lncRNAs act as miRNA sponges in the pathogenesis of DR (Table **1**).

4. LncRNA ACTS AS MIRNA SPONGE TO REGULATE TARGET GENE

4.1. LncRNA-*MEG3*

MEG3 is known as an imprinted gene, and it locates on 14q32.3. It is involved in the regulation of inflammation,

Table 1. Examples of dysregulation of lncRNAs involved in diabetic retinopathy.

LncRNA	Dysregulation	Samples	Functions and Mechanism	References
HOTAIR	Up-regulation	DR patients, DM mice, mREC cells,	Increases the cell proliferation, invasion, migration, and hyper-permeability and acts as a scaffold of histone modification complexes to inhibit the expression of VE-cadherin and increase VEGF.	[18]
ANRIL	Up-regulation	DR patients, DM mice, hRECs	Be activated by the RAS and the NF-κB pathway to bind to both p300 and EZH2 to up-regulate the expression of VEGF. Regulates the NF-κB pathway, promotes inflammation, improves vascular permeability, and declines vascular function.	[10]
MEG3	Down-regulation	DR patients, DM mice, ARPE-19 cells, Müller cells, hRECs, hRMECs, RPE.	Suppresses neovascularization, microvascular leakage, apoptosis, inflammation, proliferation, migration, and tube formation, inhibits the NF-κB signaling pathway, prevents the development of DR by reducing the expression of TGF-β1 and VEGF,	[7, 9, 68]
			MEG3*/*miRNA-34a Inhibits both inflammation and apoptosis through regulating NF-κB pathways and BAX/BCL-2 ratio by targeting *miR-34a*/SIRT1axis in hRECs.	[9]
			***MEG3*/*miR-19b*/SOCS6** Suppresses apoptosis by sponging *miR-19b* by regulating SOCS6/JAK2/STAT3 signaling in hRMECs.	[69]
			***MEG3*/*miR-204*/SIRT1 axis** Inhibits inflammation by sponging *miR-204* to elevate SIRT1, which may suppress the inflammatory cytokine secretion, including VEGF, TNF-α, IL-1β, and IL-6 in Müller cells.	[70]
			***MEG3*/*miR-93*/NRF2** Depresses apoptosis and inflammation of RPE *via miR-93*/NRF2 axis	[15]
			MEG3*/*miR-223-3p Represses the proliferation, migration, and tube formation properties by *MEG3*/*miR-223-3p* axis	[16]
MALAT1	Up-regulation	DR patients, DM mice, RF/6A cells, hRECs, hRMECs	Acts as scaffolds to chromatin-associated complexes to further modulate the expression of genes. Induces retinal microvascular dysfunction, cell proliferation, migration, tube formation, and vascular permeability. Promotes inflammatory response by increasing the expression of IL-6, TNF-α, MCP-1, and IL-1β	[19]
			MALAT1*/*miR-125b Promotes angiogenesis by targeting *miR-125b* and then activates the expression of VE-cadherin.	[71]
			MALAT1*/*miR-203a-3p Elevates HIF-1α and VEGFA levels and facilities angiogenesis in DR by sponging *miR-203a-3p*.	[72-74]
MIAT	Up-regulation	DR patients, ARPE-19 cells, DM mice, Müller cells, hRPCs, RGC-5, RF/6A	Upregulates the expression of TGF-β1 and increases cell viability, cell proliferation, migration, tube formation, inflammation, and microvascular impairment.	[75]
			MIAT*/*miR-29b Enhances vascular dysfunction, including corneal angiogenesis, retinal angiogenesis, vascular leakage, and cell apoptosis by the regulatory NF-κB/*MIAT*/*miR-29b*/SP1 network.	

(Table 1) contd….

LncRNA	Dysregulation	Samples	Functions and Mechanism	References
H19	Down-regulation		***MIAT/miR-342-3p*/CASP1** Regulates caspase-1 expression and consequent pericyte pyroptosis by sponging to *miR-342-3p*	[17]
			***MIAT/miR-150-5p*/VEGF** Upregulates the expression of VEGF by *MIAT/ miR-150-5p*/VEGF ceRNA regulatory network	[74]
		DR patients, hRECs, ARPE-19 cells	***H19/miR-19b*/SIRT1** Suppresses the expressions of inflammatory cytokines, including TNF-α, IL-1β, and IL-6, and plays a key role in HG-induced inflammatory response in ARPE-19 cells by *H19/miR-19b*/SIRT1 axis.	[76]
NEAT1	Down-regulation	DR patients, DM rats, hRECs, Müller cells	Actives TGF-β1 and VEGF, reduces cell viability, induces apoptosis, and affects antioxidant effect. ***NEAT1/miR-497*/BDNF** Inhibits apoptosis and aggravating of DR through decreasing *miR-497* and elevating BDNF by *NEAT1-miR-497*-BDNF axis.	[77] [78]

apoptosis, angiogenesis, cell proliferation, and more [25, 79-82]. In fact, *MEG3* functions as a tumor suppressor and participates in many types of cancer [80, 83]. *MEG3* may also inhibit the progression of DR [7, 82]. Compared with levels in healthy people, levels of *MEG3* in patients with DR and diabetes were significantly lower, and serum levels of VEGF and TGF-β1 were significantly higher [7]. Similar results have been observed in an acute retinal pigment epithelial cell line (ARPE-19) under HG conditions and in the retinas of mice with diabetes [7, 25]. These results support the use of *MEG3* as a marker [25, 84]. When *MEG3* is overexpressed, retinal vascular leakage, cellular capillary number, and retinal hard exudates are reduced [9]. Furthermore, overexpression of *MEG3* can decrease VEGF, IL-1β, and TGF-β1 to inhibit the development of DR [7, 9, 68]. Increasing the expression of *MEG3* seems important to improving treatment options for DR. However, the function and mechanism of *MEG3* in DR remain unclear and should be studied more.

Recent studies have reported that *MEG3* can bind to many different miRNAs to regulate the expression of target genes in DR. In studies, *MEG3* reduced HG-induced inflammation, apoptosis, and cell dysfunction by sponging different miRNAs, such as *miR-34, miR-19, miR-204, miR-93,* and *miR-223-3p* (Fig. **3**) [9, 15, 69, 70, 85].

4.1.1. MEG3/miRNA-34a

MEG3 could serve as a positive regulator of silent information regulator factor-related enzyme 1 (SIRT1), one of the histone deacetylases. Tong *et al.* reported that the expression level of *MEG3* decreased prominently in ARPE-19 cells under HG conditions, whereas the level of *miR-34a* increased and the levels of SIRT1 decreased. SIRT1 is a direct target of *miR-34a,* as verified by the dual-luciferase reporter assay [9], and *miR-34a* is involved in the processes of apoptosis and inflammation [86, 87]. The expression of *miR-34a* is significantly upregulated in diabetes [88]. The activation of SIRT1 could lead to downregulation of NF-κB as well as some proinflammatory cytokines (*e.g.*, IL-1β, TNF-α, and IL-6) [89]. SIRT1 may be able to inhibit inflammatory responses, and it can prevent oxidative stress damage in retinal cells. Therefore, SIRT1 could affect the pathogenesis and progression of DR [89, 90].

Tong *et al.* explored the deeper mechanism for *MEG3* regulation of the NF-κB pathway in DR [9]. They discovered that the protein level of SIRT1 was reduced in ARPE-19 cells under HG conditions, as was the protein level of inhibitor of nuclear factor kappa-B (IκB). Conversely, the protein levels of phosphorylated IκB (p-IκB) and phosphorylated p65 (p-p65) increased. IκB is a pivotal regulator in the NF-κB pathway, which plays an important role in promoting apoptosis and releasing inflammatory cytokines [91, 92]. When IκB and NF-κB form a heterodimer, NF-κB remains inactive. After IκB is phosphorylated, degradation of IκB occurs, releasing free and active NF-κB [93]. Thus, increasing levels of p-IκB and p-p65 may indicate activation of the NF - κ B signaling pathway. HG treatments can activate the NF-κB signaling pathway by decreasing SIRT1; conversely, upregulating SIRT1 would inhibit the NF-κB signaling pathway, possibly through overexpression of *MEG3* and knockdown of *miR-34a*. However, Tong *et al.* found that the ratio between B cell lymphoma/leukemia-2 (BCL-2) and BCL2 associated X (BAX) (BCL-2/BAX) increased when *MEG3* was overexpressed or *miR-34a* was knocked down [9]. BCL-2 is an important anti-apoptotic protein, and BAX is a pivotal proapoptotic protein that can promote apoptosis [94]. A higher BCL-2/BAX ratio implies that apoptosis would decrease. All these data suggest that *MEG3* could inhibit inflammation as well as apoptosis and that the inhibition could be accomplished through the *miR-34a*/SIRT1 axis, which can regulate the NF-κB pathway and the BCL-2/BAX ratio as the end result of DR processes (Fig. **3**) [9].

*4.1.2. MEG3/miR-204/*SIRT1 *Axis*

Another target of *MEG3*, *miR-204*, also targets SIRT1. Tu *et al.* explored the level of *MEG3* in HG-treated human Müller (MIO-M1) cells and mice with DR [70]. It was

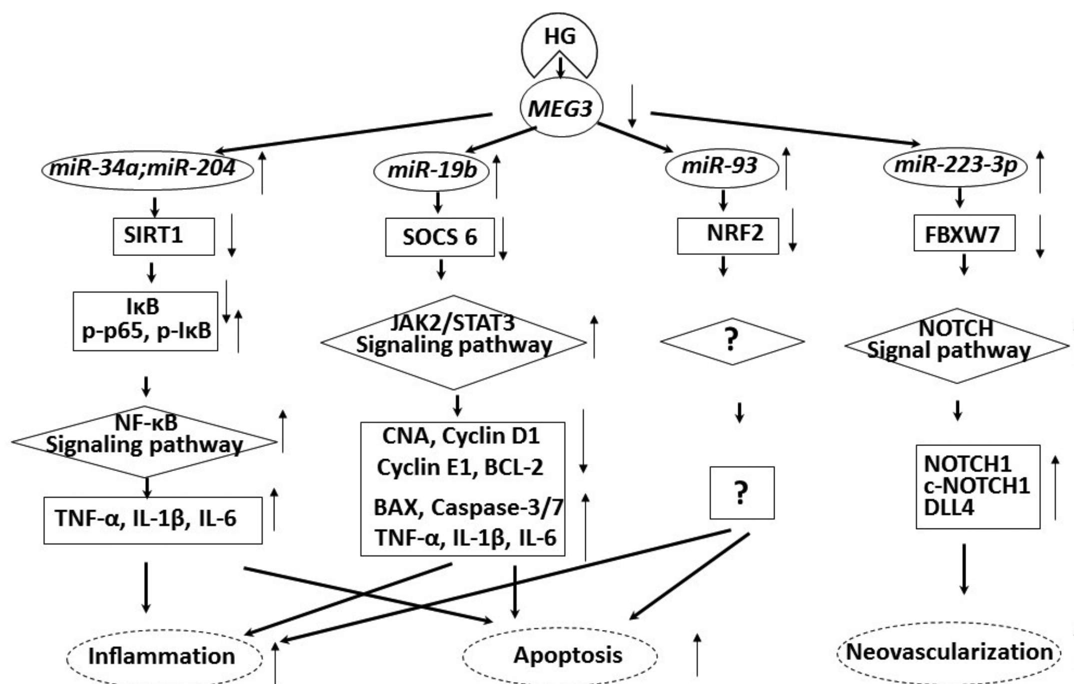

Fig. (3). *MEG3* regulates target genes by sponging miRNAs axes in the progression of DR. The expression of *MEG3* decreases prominently under HG conditions, whereas the expressions of *miR-34a*, *miR-19b*, *miR-93* and *miR-223-3p* increase, and the levels of SIRT1, SOCS6, NRF2, and FBXW7 decrease. Signaling pathways of NF-κB, JAK2/STAT3 and NOTCH would be stimulated, which could affect many factors to activate the inflammation, apoptosis, and neovascularization in the progression of DR.

found that, when *MEG3* expression decreased, *miR-204* expression increased, and the SIRT1 protein level was downregulated. *MEG3* could sponge *miR-204* to regulate SIRT1, which might affect the secretion of inflammatory cytokines, including VEGF, IL-1β, TNF-α, and IL-6 (Fig. **3**) [70].

*4.1.3. MEG3/miR-19b/*SOCS6

MEG3 could trigger apoptosis *via* the suppressor of cytokine signaling 6 (SOCS6)/JAK2/STAT3 signaling pathway, which is regulated by *MEG3* sponging of *miR-19b* [69]. This mechanism might be a novel therapeutic strategy for DR. *miR-19b* has many important functions in HG-induced human retinal microvascular endothelial cells (hRMECs). For example, miR-19b can depress cell proliferation and aggravate apoptosis and inflammation. Xiao *et al.* reported that *miR-19b* expression was highly elevated in HG-induced hRMECs, whereas SOCS6 was obviously decreased. Luciferase reporter assay confirmed that *miR-19b* could target SOCS6. Overexpressed *miR-19b* has inhibited not only cell viability but also the protein levels of proliferation-related molecules, such as cell nucleolar antigen (CNA). Inhibitory effects also have been observed on BCL-2, cyclin E1, and cyclin D1 but not on caspase-3/7 or BAX. The activity of caspase-3, caspase-7, and BAX increased significantly with *miR-19b* overexpression, which markedly promoted the cell apoptosis rate. Conversely, when *miR-19* was markedly elevated by *miR-19* mimics, the expressions of IL-6, TNF-α, and IL-1β increased [69]. Together, these findings showed that *MEG3* is able to suppress the processes of inflammation and apoptosis (Fig. **3**). As the *MEG3*/*miR-19b*/SOCS6 axis regulates the JAK2/STAT3 signaling pathway, *MEG3* would be useful for the treatment of DR [69].

*4.1.4. MEG3/miR-93/*NRF2

The *miR-93*/ nuclear factor-erythroid 2 related factor 2 (NRF2) axis is crucial for restraining *MEG3*-related inflammation and apoptosis in the retinal pigmented epithelium (RPE). Rong *et al.* reported that levels of both *MEG3* and NRF2 were decreased, whereas levels of *miR-93* were increased in patients with DR. The same results have been observed *in vitro* [15]. Cell proliferation was augmented when MEG3 or NRF2 was overexpressed, whereas inflammation and apoptosis were reduced. However, when *miR-93* was overexpressed, the opposite result was observed: cell proliferation was depressed, and apoptosis was augmented. A dual-luciferase reporter assay proved the target relationship among *MEG3*, *miR-93*, and NRF2. This study showed that *MEG3* reduces inflammation and apoptosis, a finding that is useful for understanding the genesis and development of DR (Fig. **3**) [15].

4.1.5. MEG3/miR-223-3p

MEG3 can regulate *miR-223-3p* expression [16, 85]. Fan *et al.* found that overexpression of *MEG3* could inhibit neovascularization in DR *in vivo* and *in vitro* [16]. For example, when MEG3 levels were enhanced, cell proliferation, migration, and tube formation were suppressed *in vitro,* and retinal vascular leakage was reduced *in vitro* [16]. Results of luciferase reporter assays demonstrated the direct binding between *MEG3* and *miR-223-3p*. Furthermore, the assays showed that *MEG3* overexpression and *miR-223-3p* inhibition could stimulate FBXW7, the negative regulator for the NOTCH1 signal pathway, which could suppress many protein factors, such as NOTCH1, c-NOTCH1, and DLL4, in the signal pathway, thus preventing neovascularization in

DR. Conversely, *MEG3* knockdown or *miR-223-3p* mimic could produce the opposite effect; FBXW7 was inhibited, the NOTCH1 signal pathway was activated, and neovascularization in DR was promoted (Fig. **3**) [16].

4.2. LncRNA-*MALAT1*

MALAT1 acts as a scaffold to recruit chromatin remodeling complexes that regulate gene expression in nuclei, as mentioned in section 3.2 [19, 53, 54]. In fact, *MALAT1* can bind to different miRNAs to suppress the expression of corresponding target genes in the cytoplasm. *MALAT1* can accelerate angiogenesis through various miRNAs, multiple miRNA axes, and signaling pathways, such as miR125b and miR-203a-3p (Fig. **4**) [71, 72, 95]. However, details about how *MALAT1* affects the pathogenesis of DR remain unclear.

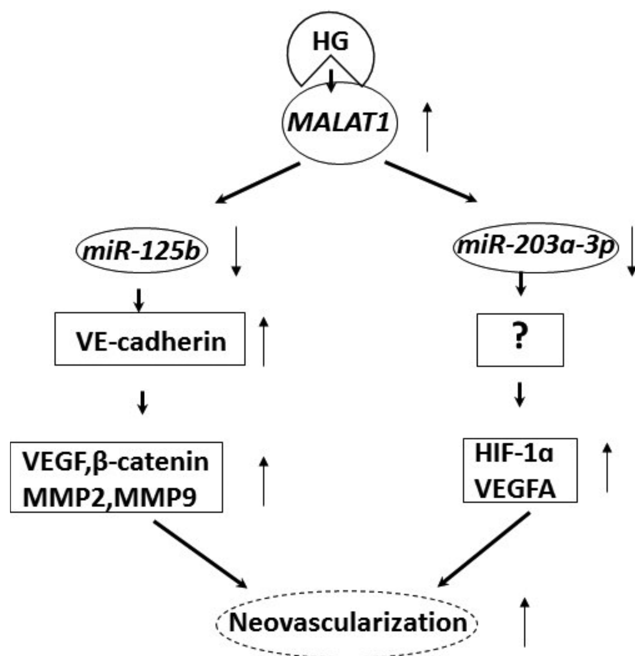

Fig. (4). *MALAT1* regulates target genes by sponging miRNAs axes in the neovascularization progression of DR. The expression of *MALAT1* is significantly augmented under HG, whereas the levels of *miR-125b* and *miR-203a-3p* are reduced, which could stimulate the levels proteins associated with neovascularization, such as VEGF and so on. MALAT1 can accelerate angiogenesis in DR.

4.2.1. MALAT1/miR-125b/ VE-cadherin

MALAT1 can regulate the expression of *miR-125b* and VE-cadherin to promote neovascularization in DR [71]. Liu *et al.* found that levels of *MALAT1* and VE-cadherin were significantly augmented in hRMECs treated with HG, whereas the level of *miR-125b* was reduced drastically [71]. Evidence indicates that *miR-125b* is associated with the progression of DR. When *miR-125b* was knocked down, target genes were up-regulated, promoting tube formation in endothelial cells in DR [71, 72, 96, 97]. *MiR-125b* is a target of *MALAT1*, and *MALAT1* can directly sponge *miR-125b* to antagonize the *miR-125b* inhibitory effect on VE-cadherin [71]. Furthermore, Liu *et al.* found that *MALAT1* deletion suppressed the expressions of proteins associated with neovascularization, such as VE-cadherin. They speculated that *MALAT1* might promote angiogenesis. These findings suggest that *MALAT1* might be a new target for DR therapy and that antagonists of *MALAT1* or *miR-125b* mimics might prevent neovascularization in DR [71] (Fig. **4**).

4.2.2. MALAT1/miR-203a-3p

MALAT1 can facilitate angiogenesis in DR by sponging *miR-203a-3p* to augment levels of HIF1α and VEGFA. Yu *et al.* reported that expressions of HIF-1α, VEGFA, and *MALAT1* increased in the retinas of rats with diabetes and in hRMECs treated with HG; however, the level of *miR-203a-3p* decreased [72]. *MALAT1* knockdown or overactive expression of *miR-203a-3p* could inhibit the angiogenesis of HRMECs induced by HG [72].

The targeting of *MALAT1* by *miR-203a-3p* has been confirmed by a dual-luciferase assay. Moreover, *miR-203a-3p* affects the regulation of *MALAT1* on migration and tube formation. The *MALAT1*/*miR-203a-3p* axis may be in charge of the procession of DR [72]. These data demonstrate that *MALAT1* is important in the treatment of DR (Fig. **4**).

4.3. LncRNA-*MIAT*

LncRNA myocardial infarction associated transcript (*MIAT*) was first reported in mitotic progenitors and postmitotic retinal precursor cells [98], but its dysregulating effect has subsequently been identified in many diseases, including microvascular dysfunction, diabetes, and DR [73, 74, 99-101]. Higher *MIAT* levels are present in the diabetic *versus* the normal healthy retina as well as in the fibrovascular membranes of patients with diabetes and ARPE-19 cells under HG conditions [73, 74]. *MIAT* is reportedly involved in regulating the function of vascellum in diabetes [102]. When *MIAT* was knocked down in an *in vivo* experiment, impairment of micro vascularization was reduced; moreover, in an *in vitro* experiment, tube formation, cell migration, and cell proliferation were inhibited [74]. *MIAT* knockdown could also suppress retinal inflammation in animal models of diabetes. Accumulating evidence shows that *MIAT* may be crucial to regulating the process of DR (Fig. **5**).

*4.3.1. MIAT/miR-29b/*SP1

MIAT can act as several types of miRNA sponges to play functions in the pathogenesis of DR. Zhang *et al.* showed that *MIAT*, p-p65, and SP1 were enhanced remarkably as *miR-29b* expression decreased in a murine model of streptozocin (STZ)-induced diabetes and HG-stimulated rat retinal Müller cells (rMC-1) [75]. They demonstrated that the *MIAT* promoter was bound by NF-κB, and the binding activation was significantly increased in HG-treated rMC-1 cells. In addition, the cell survival rate increased when *MIAT* was suppressed, whereas apoptosis decreased; *miR-29b* expression was enhanced significantly, and the increased expression of SP1 induced by HG conditions was decreased. Conversely, when *MIAT* was overexpressed or *miR-29b* was knocked down, cell apoptosis and SP1 expression were significantly promoted [75].

SP1 is directly targeted by *miR-29b* and represses the expression of *miR-29b* [103, 104]. Zhang *et al.* found that

MIAT harbors *miR-29b* and inhibits the expression of *miR-29b* and SP1. The specific regulatory network, NF-κB/*MIAT*/*miR-29b*/SP1 network regulating cell apoptosis, appears to be important for the clinical therapy of DR (Fig. **5**) [75].

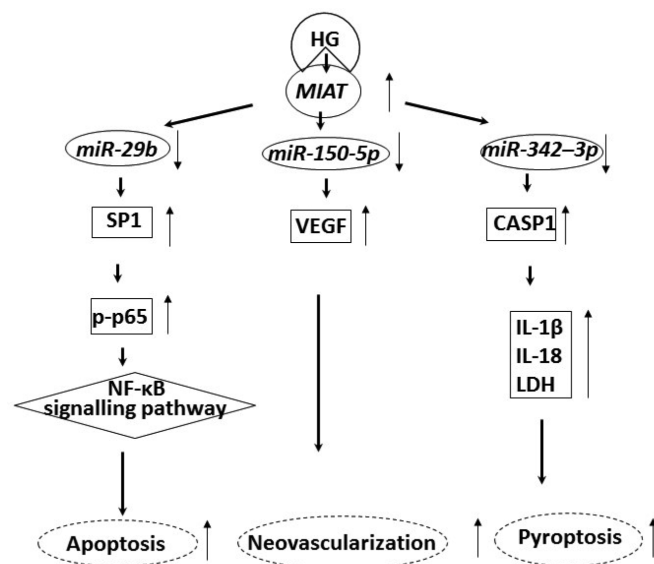

Fig. (5). *MIAT* regulates target genes by sponging miRNAs axes in the progression of DR. The expression of *MIAT* is accelerated by HG conditions, and the target genes of *miR-29b, miR-150-5p*, and *miR-342-3p* are inhibited. As a result, the levels of downstream proteins SP1, VEGF, and CASP1 are increased, ultimately promoting the development of apoptosis, neovascularization and pyroptosis in the progression of DR.

*4.3.2. MIAT/ miR-150-5p/*VEGF

MIAT functions on angiogenesis by regulating *miR-150-5p*/VEGF in DR. Yan *et al.* confirmed the target relationship between *miR-150-5p* and *MIAT in vitro* by luciferase assay. When they overexpressed a *miR-150-5p* mimic by intravitreal injection into both diabetic and non-diabetic rats *in vivo*, the *MIAT* levels decreased considerably. However, when the expression of *miR-150-5p* was significantly reduced by injection of a *miR-150-5p* antagonist, the *MIAT* levels increased notably [74]. RNA immunoprecipitation showed that *miR-150-5p* targeted *MIAT* in a manner dependent on Ago2, which is a key constituent of the RNA-induced silencing complex (RISC). It is essential for RISC to bind miRNA, forming complexes to regulate the transcription of mRNA [105]. Knockdown of Ago2 increased *MIAT* levels but lessened the stability of *miR-150-5p*. *MIAT* overexpression significantly upregulated the VEGF level, whereas *miR-150-5p* overexpression significantly decreased the VEGF level. Together, the results demonstrated interactivity among lncRNA-*MIAT*, VEGF, and *miR-150-5p*. The role of the *MIAT*/*miR-150-5p*/VEGF network in ocular angiogenesis might ultimately facilitate the development of lncRNA-directed diagnostics and therapeutics for DR (Fig. **5**) [74].

*4.3.3. MIAT/miR-342-3p/*CASP1

MIAT can function as a *miR-342-3p* sponge to regulate the transcription of its target gene, caspase-1(CASP1), which could affect the pyroptosis of pericyte cells. Pyroptosis, a newly discovered programmed death of inflammatory cells, relies on the activation of a variety of caspases [106, 107]. CASP1 is important among the caspases mostly for its regulation of pyroptosis in retinopathy [108-110]. Yu *et al.* applied advanced glycation end product modified bovine serum albumin (AGE-BSA) to simulate human retinal pericytes (hRPCs) to build a DR environment [17]. The occurrence of pyroptosis was also identified, which reduced cell viability and increased the levels of *MIAT* and CASP1; conversely, *miR-342-3p* levels decreased in AGE-BSA-treated hRPCs. A luciferase reporter assay showed that *miR-342-3p* targeted *MIAT* and CASP1 targeted miR-342-3p in HRPCs. *MIAT* exerted a negative regulatory effect on miR-342-3p. Knockdown of *MIAT* by siRNA or overexpression of *miR-342-3p* using a *miR-342-3p* mimic both decreased the level of CASP1 and inhibited inflammation [17]. Thus, *MIAT* promotes pyroptosis *via* the *MIAT*/miR-342-3p/CASP1 signaling pathway. Inhibited expression of *MIAT* alleviates the process of pyroptosis, which may be beneficial for providing new strategies to treat DR.

4.4. LncRNA *H19* and *H19*/*miR-19b*/SIRT1 Axis

The *H19*/*miR-19b*/SIRT1 axis functions as a core regulatory pathway in the inflammatory response that is induced by HG conditions [76]. *H19* is poorly expressed in HRECs with HG levels [41]. *H19* could sponge *miR-93* to mediate the expressions of IL-6, TNF-α, and IL-1β in retinal epithelial cells under HG conditions [14]. Luo *et al.* explored the interaction among *H19*, *miR-19b*, and SIRT1 in ARPE-19 cells with HG. It was found that the expressions of both lncRNA *H19* and SIRT1 decreased, whereas the expression of *miR-19b* increased. At the same time, the transcription levels of IL-6, TNF-α, and IL-1β were all significantly augmented when *miR-19b* expression was added in ARPE-19 cells with HG conditions. Furthermore, when *miR-19b* was knocked down, the opposite effects occurred. Thus, the expressions of inflammatory cytokines can be regulated by *miR-19b* in ARPE-19 cells. A dual-luciferase reporter assay confirmed that *H19* directly targeted *miR-19b* to control the expression of SIRT1. Depressing *miR-19b* could increase SIRT1, thus reducing the inflammatory response (Fig. **6**) [76].

4.5. LncRNA *NEAT1* and *NEAT1*/*miR-497*/BDNF Axis

Nuclear enriched abundant transcript 1 (*NEAT1*) is found to be involved in many diseases, including tumors [111]. *NEAT1* also functions as a miR-sponge and regulates downstream molecules [112-115]. Studies have shown that aberrant expression of *NEAT1* occurs in DR [77, 78]. The level of *NEAT1* increased greatly in the serum of patients with DR and in the retina of Sprague-Dawley rats with streptozocin-induced diabetes. Similar results were obtained *in vitro* when increased *NEAT1* developed in HRECs under HG treatment [77]. Flow cytometry results revealed the acceleration of cell apoptosis with higher levels of *NEAT1* and have shown that loss of *NEAT1* can inhibit cell apoptosis induced by HG stimulation in HRECs. Western blot analysis has shown that BCL-2 expression was increased, and BAX expression was decreased when *NEAT1* was lost [77].

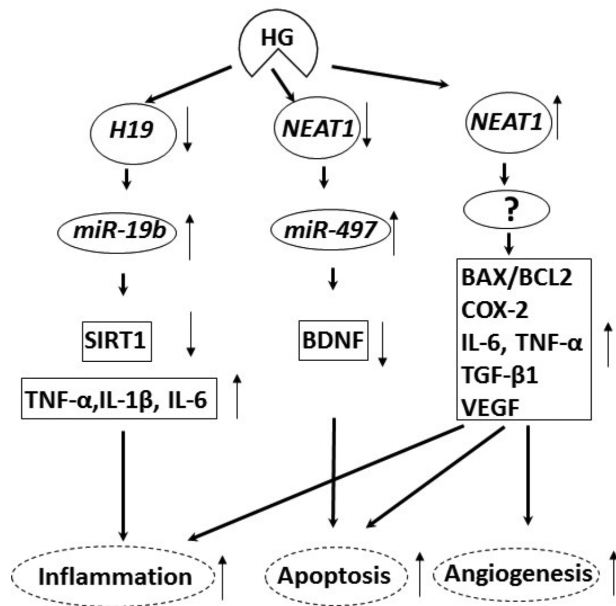

Fig. (6). *H19* and *NEAT1* regulate target genes by sponging miRNAs axes in the progression of DR. The *H19*/*miR-19b*/SIRT1 axis regulates the inflammatory response under HG conditions. The expression of *H19* is decreased, miR-19b is increased, and the level of SIRT1 is inhibited. The inflammation is promoted with the augmenting levels of IL-6, TNF-α, and IL-1β. Some studies showed that the levels of *NEAT1* are higher with HG stimulation, and as a result, BAX/BCL2, COX-2, IL-6 and so on can be activated. Inflammation, apoptosis and angiogenesis are accelerated. On the contrary, some researchers found the levels of *NEAT1* decreased under HG treatment; apoptosis was accelerated by *NEAT1*/*miR-497*/BDNF axis.

In addition to its effects on apoptosis, *NEAT1* can also regulate inflammation and angiogenesis. Shao *et al.* observed that protein levels of cyclooxygenase-2(COX-2), IL-6, and TNF-α decreased when *NEAT1* was knocked down. At the same time, both VEGF and TGF-β1were repressed by the silencing of *NEAT1*. Thus, COX-2, IL-6, TNFα, TGF-β1, and VEGF can be activated by *NEAT1*, which triggers oxidative stress, inflammation, and angiogenesis [77]. Although *NEAT1* is considered an epigenetic modulator in DR, the specific role of *NEAT1* in the occurrence of DR remains unclear, and little research is available. Li found that *NEAT1* could positively regulate the expression of brain-derived neurotrophic factor (BDNF) by interacting with *miR-497* [78]. Li reported that the levels of *NEAT1* and BDNF markedly decreased, along with a remarkable increase in *miR-497* expression, in Müller cells under HG treatment. The association between *NEAT1* with *miR-497* was analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) and RNA pull-down assays. These results confirmed that *NEAT1* might interact with *miR-497*. Luciferase report gene assays also indicated that BDNF was negatively regulated by *miR-497*. Furthermore, apoptosis of Müller cells promoted by HG treatment was attenuated, and cleaved caspase-3 expression was reduced when *NEAT1* was overexpressed. However, when *NEAT1* expression decreased, apoptosis increased. Broadly, when *NEAT1* is downregulated, the expression of BDNF decreases as a result of the augmented expression of *miR-497* under HG treatment. As a result, apoptosis is accelerated, and DR symptoms become more serious (Fig. **6**) [78]. Both Shao *et al.* and Li reported that *NEAT1* regulated cell apoptosis *in vitro* and that dysregulated levels of *NEAT1* were able to change the cell apoptosis rate [77, 78]. However, there are some differences between these studies. Shao *et al.* showed that the *NEAT1* levels increased in HRECs under HG treatment and that *NEAT1* knockdown would reduce cell apoptosis [77]; Li observed that *NEAT1* levels decreased in Müller cells under HG treatment and that overexpression of *NEAT1* would inhibit cell apoptosis [78]. What is the reason for the different results? These studies used different cell lines in the retina. What if *NEAT1* targets many different kinds of miRNAs and participates in many different pathways? What is the more detailed mechanism? And what is the truth? Additional studies are needed to clarify the function of *NEAT1* in DR.

LncRNAs can sponge different miRNAs and play a crucial function in mediating target molecules and modulating various pathways in angiogenesis, inflammation, and apoptosis in the processes of DR (Fig. **7**) [5, 116, 117]. Wu *et al.* identified 305 differentially expressed lncRNAs, and 17 differentially expressed miRNAs in the diabetic retina samples compared to controls [12]. Cao *et al.* identified other ceRNA networks that comprised 410 lncRNAs, 35 miRNAs, and 122 mRNAs, in which the lncRNA OIP5-AS1-miR-449c-MYC axis affected apoptosis of HG-treated HRECs [13]. Though much research about the roles of protein-coding genes or miRNAs in the pathogenesis of DR has been conducted, the mechanism of action within the lncRNA-miRNA-mRNA network in DR remains unclear [12]. The action and mechanism performed by the lncRNA-miRNA-mRNA network in the pathogenesis of DR need more research [13, 76].

5. DISCUSSION

DR is a complex and multifactorial complication of diabetes, leading to severe vision impairment and blindness. Although a variety of treatments for DR are currently available, none of them are fully effective. It has now become evident that non-coding RNAs, especially lncRNAs and miRNAs, are dysregulated in DR. Indeed, dysfunctional lncRNAs and miRNAs have a critical role in developing DR. Recent studies have shown that lncRNAs can interact with the protein, DNA, and RNA. LncRNA can modify the state of chromatin to modulate the expression of a target gene by acting as a scaffold for protein complexes. In addition, lncRNAs may fulfill the function of ceRNA *via* a miRNA sponge, decreasing the regulatory activity of miRNA on mRNAs. Thus, lncRNAs are important epigenetic regulators of the expression of target genes. A growing number of lncRNAs are now implicated in the pathogenesis of DR, including in the processes of angiogenesis, inflammation, and apoptosis. However, the mechanism of action for lncRNA participation in the pathogenesis of DR is quite complex. The effect of the lncRNA-miRNA-mRNA network in the pathogenesis of DR is still not sufficiently understood.

The complexity among lncRNA, miRNA, and mRNA is embodied in the overlapping ways that the network participates in the progression of DR. In one interaction, a kind of

lncRNA binds various miRNAs to regulate one mRNA. For example, *MEG3* can regulate the transcription of SIRT1 by both *MEG3*-miR34 and *MEG3-miR-204* to affect the processes of inflammation and apoptosis in DR [9, 70]. In another interaction, different lncRNAs act as different miRNA sponges to target a single mRNA (*e.g*., the *MEG3*/*miR-34a*/SIRT1axis or the *H19*/*miR-19b*/SIRT1 axis) [9, 76]. In a third interaction, distinct lncRNAs target the same miRNA but regulate different mRNAs. For example, overexpression of *MEG3* can inhibit *miR-19b* transcription and up-regulate the expression of SOCS6, but overexpression of *H19* can also inhibit *miR-19b* transcription and increase the expression of SIRT1. A fourth interaction involves different lncRNAs binding to various miRNAs to target diverse mRNAs and regulate multiple signaling pathways in the origination and development of DR. Hence, the complicated lncRNA-miRNA-mRNA network exhibits diversified mechanisms in the pathogenesis of DR [118]. Fortunately, in this era of rapid development in computational methodology, computational approaches are emerging to simulate and understand various chemical and biological systems. These approaches could bring revolutionary changes to the exploration of the lncRNA-miRNA-mRNA network [119, 120]. The challenge is to improve our understanding of the characteristics of lncRNAs in DR to address the gaps in the basic knowledge regarding lncRNA in the clinical practice of DR [121].

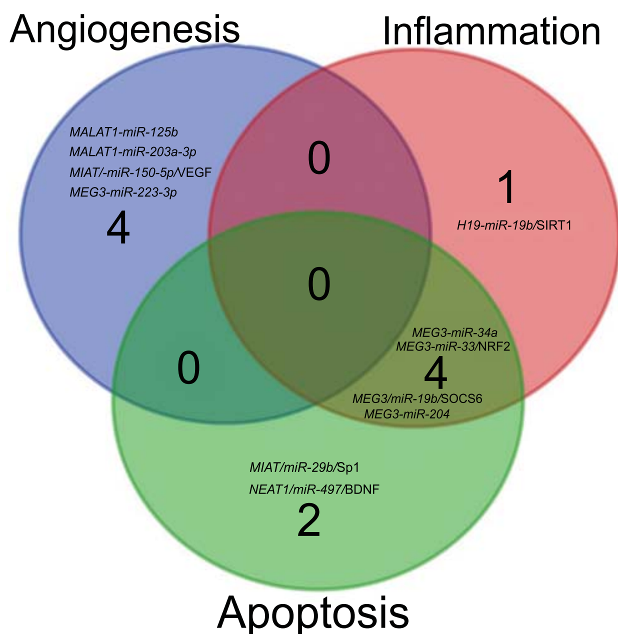

Fig. (7). LncRNAs play crucial functions in angiogenesis, inflammation, and apoptosis in the process of DR by sponging different miRNAs and regulating responsive target genes. The Venn diagram is drawn using an online tool. (http://bioinformatics.psb.ugent.be/beg/tools/venn-diagrams). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

CONCLUSION

Therapies that are based on the lncRNA-miRNA-mRNA network may have many advantages (*e.g*., the use of antagonists or mimics to inhibit or enhance the regulatory function of the network). Knockdown of specific lncRNAs has been confirmed in animal models but rarely explored in clinical trials as a way to innovate the treatment of DR [10, 18, 19, 59]. LncRNA knockdown is a promising strategy for lncRNA-specific drug discovery in DR; more efforts are needed to investigate this field and identify new diagnostic markers and novel therapeutic targets for DR.

LIST OF ABBREVIATIONS

ANRIL = Antisense non-coding RNA in the INK4 locus
ARPE-19 = Acute retinal pigment epithelial cell line
BAX = Bcl-2 associated x protein
BCL-2 = B cell lymphoma/leukemia-2
BDNF = Brain-derived neurotrophic factor
BRB = Blood-retinal barrier
CAN = Cell nucleolar antigen
CASP1 = Caspase-1
ceRNA = Competing endogenouse RNA
CoREST = Corepressor for repressor element-1 silencing transcription factor
COX-2 = Cyclooxygenase-2
DM = Diabetes mellitus
DR = Diabetic retinopathy
EED = Embryonic ectoderm development
EZH2 = Enhancer of zeste 2
H19 = Imprinted maternally expressed transcript
H3K27 = H3 Lys 27
H3K27me3 = H3K27 trimethylation
H3K4 = H3 Lys 4
H3K4me3 = H3K4 trimethylation
HG = High glucose
HIF1 α = Hypoxia inducible factor 1 α
HOTAIR = HOX antisense intergenic RNA
hRECs = Human retinal endothelial cells
hRMECs = Human retinal microvascular endothelial cells
hRPCs = Human retinal pericytes
IL = Interleukin
IκB = Inhibitor of nuclear factor kappa-B
KO = Knockout
LDH = Lactate dehydrogenase
lncRNA = Long noncoding RNA
LSD1 = Lysine-specific demethylase 1
MALAT1 = Metastasis-associated lung adenocarcinoma transcript 1
MCP-1 = Monocyte chemotactic protein-1
MEG3 = Maternally expressed gene 3
MIAT = Myocardial infarction associated transcript
miRNA = microRNA
MMP = Matrix metalloproteinase
mREC = Mouse retinal endothelial cells
ncRNAs = Non-protein-coding RNAs
NEAT1 = Nuclear enriched abundant transcript 1
NF-κB = Nuclear factor kappa-B
NRF2 = Nuclear factor-erythroid 2 related factor 2
PRC2 = Polycomb repressive complex 2
qRT-PCR = Quantitative real-time polymerase chain reaction

RPE	=	Retinal pigmented epithelium
SIRT1	=	Silence information regulator factor related enzymes 1
SOCS6	=	Suppressor of cytokine signaling 6
SUZ12	=	Suppressor of zeste 12 homolog
TGF-β1	=	Transforming growth factor-β1
TNF-α	=	Tumor necrosis factor
VE-cadherin	=	Vascular endothelial-cadherin
VEGF	=	Vascular endothelial growth factor
VEGFA	=	Vascular endothelial growth factor A

AUTHORS' CONTRIBUTION

Peng Chen conceptualized the project. Zhaoxia Song wrote the manuscript. Chang He and Jianping Wen contributed to the figures. Jianli Yang summarized the tables. All authors contributed significantly and agreed with the content of the manuscript.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

P.C. is sponsored by the "Changbai Mountain Scholar" distinguished professor reward program of the Department of Education of Jilin Province.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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