**Genetics**

### Aberrant Expression of Long Noncoding RNAs in Early Diabetic Retinopathy

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**PURPOSE.** Long noncoding RNAs (IncRNAs) are broadly classified as transcripts longer than 200 nucleotides. IncRNA-mediated biology has been implicated in a variety of cellular processes and human diseases. Diabetic retinopathy (DR) is one of the leading causes of blindness. However, little is known about the role of IncRNAs in DR. The goal of this study aimed to identify IncRNAs involved in early DR and characterize their roles in DR pathogenesis.

**METHODS.** We established a mouse model of streptozotocin (STZ)-induced diabetes, and performed IncRNA expression profiling of retinas using microarray analysis. Based on the Pearson correlation analysis, an IncRNA/mRNA coexpression network was constructed. Gene ontology (GO) enrichment and KEGG analysis of IncRNAs–coexpressed mRNAs was conducted to identify the related biological modules and pathologic pathways. Real-time PCR was conducted to detect the expression pattern of IncRNA in the clinical samples and the RF/6A cell model of hyperglycemia.

**RESULTS.** Approximately 303 IncRNAs were aberrantly expressed in the retinas of early DR, including 214 downregulated IncRNAs and 89 upregulated IncRNAs. GO analysis indicated that these IncRNAs–coexpressed mRNAs were targeted to eye development process (ontology: biological process), integral to membrane (ontology: cellular component), and structural molecule activity (ontology: molecular function). Pathway analysis indicated that IncRNAs–coexpressed mRNAs were mostly enriched in axon guidance signaling pathway. In addition, MALAT1, a conserved IncRNA, was significantly upregulated in an RF/6A cell model of hyperglycemia, in the aqueous humor samples, and in fibrovascular membranes of diabetic patients.

**CONCLUSIONS.** IncRNAs are involved in the pathogenesis of DR through the modulation of multiple pathogenetic pathways. MALAT1, a conserved IncRNA, may become a potential therapeutic target for the prognosis, diagnosis, and treatment of DR.

Keywords: long noncoding RNA, microarray analysis, diabetic retinopathy, biomarker

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**Diabetic retinopathy (DR)** is a severe complication of diabetes.\(^1\) Approximately one-third of diabetic patients have signs of DR and approximately one-tenth of patients have the vision-threatening phases of retinopathy, such as diabetic macular edema and proliferative retinopathy.\(^2\) The pathogenesis of DR is multifactorial. Risk factors, such as poor glycemic control, longer diabetes duration, hypertension, hyperlipidemia, and albuminuria have been implicated in the initiation and progression of DR.\(^3,4\) To date, several candidate genes, including aldose reductase, VEGF receptor for advanced glycation end products gene, angiotensin I converting enzyme, methylene-tetrahydrofolate reductase, glucose transporter, plasminogen activator inhibitor 1, \(\alpha2\) integrin, and apolipoprotein E, are found to be associated with DR susceptibility, suggesting the role of genetic factors in shaping the susceptibility to DR.\(^5,6\)

The mammalian genome is transcribed in a complex manner, including the production of thousands of long noncoding RNAs (IncRNAs).\(^7\) IncRNAs are defined as the transcripts of more than 200 nucleotides that structurally resemble mRNAs but do not encode proteins. IncRNAs participate in a variety of biological processes, such as chromosome imprinting, epigenetic regulation, cell-cycle control, transcription, translation, splicing, and cell differentiation.\(^7\) Misregulation of IncRNAs is associated with the susceptibility to several human diseases, including cancers, cardiovascular diseases, and neurological diseases.\(^8\) Genome-wide association studies (GWAS) have revealed that only 7% of diseases or trait-associated single-nucleotide polymorphisms (SNPs) reside in the protein-coding regions, whereas 43% of trait/disease-associated SNPs are found outside of protein-coding genes,\(^9\) suggesting that noncoding RNA alteration could affect the genetic susceptibility to DR. Thus, identifying DR-related IncRNAs contributes to better understanding the complex molecular mechanisms of DR pathogenesis.

To reveal the potential role of IncRNAs in DR, we performed IncRNA expression profiling and compared IncRNA expression differences between diabetic retinas and nondiabetic retinas using microarray analysis. The result showed that 303 IncRNAs were aberrantly expressed in diabetic retinas. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis suggested that these differentially expressed IncRNAs may be involved in DR pathogenesis through modulating multiple pathologic signaling pathways. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), a highly conserved IncRNA, was abnormally expressed in an RF/6A cell...
Aberrant Expression of lncRNAs in Early DR

model of hyperglycemia, in the fibrovascular membranes (FVMs), and in the aqueous humor samples of diabetic patients, suggesting its potential application as a biomarker of the prognosis and diagnosis of DR. To our knowledge, this is the first direct, in-depth investigation on IncRNA expression profiling of DR, providing a novel insight into the molecular mechanisms of DR pathogenesis.

MATERIALS AND METHODS

Diabetic Mice Model

All experimental animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and approved by the Animal Care and Use Committee of Nanjing Medical University. Diabetes was induced chemically in 8-week-old C57BL/6 mice. Mice received an intraperitoneal injection of 50 mg/kg streptozotocin (STZ) dissolved in sodium citrate buffer (0.01 M, pH 4.5) on 5 successive days. Control animals received an injection of equal volume of citrate buffer. Blood glucose levels were measured immediately before STZ injection, or 2 days, 1 week, 1 month, or 2 months after STZ injection. Animals with blood glucose levels higher than 250 mg/dL were deemed as having diabetes.10

Electroretinogram

Before being euthanized for morphological and biochemical analyses, the experimental mice were subjected to analysis by electroretinogram (ERG) to evaluate the change in retinal electrical activity. Briefly, mice were dark-adapted overnight. Pupils were fully dilated using 1% tropicamide solution (Alcon, Fort Worth, TX). ERG responses were recorded from both eyes using the platinum wire corneal electrodes, forehead reference electrode, and ground electrode in the tail. ERG waveforms were recorded with a bandwidth of 0.3 to 500 Hz and samples at 2 kHz by a digital acquisition system. Statistics were shown as mean ± SEM amplitudes of A-, B-, or oscillatory potential (OP) wave of each treatment group.11

Microarray Profiling

Total RNAs were isolated from the retinas of diabetic mice 2 months after STZ injection (n = 9) or age-matched and sex-matched wild-type mice (n = 9) using TRIzol reagent (Invitrogen, Carlsbad, CA), respectively. Three individuals were pooled together as a biological repeat to further eliminate the individual difference. Microarray profiling was performed using Agilent Mouse Gene Expression Microarrays (Product Number G4852A; Agilent Technologies, Santa Clara, CA), including 39,430 Entrez Gene RNAs and 16,251 long intergenic RNAs (lncRNAs). Briefly, 10 μg of total RNAs were labeled using the Superscript Plus Direct cDNA labeling system (Invitrogen), and then hybridized to the chip. Microarrays were scanned using an Agilent scanner, and microarray data were extracted using Agilent Feature Extraction software FE10.5. The data quality was assessed using GeneSpring GX software (Agilent Technologies). Real-time PCR

Total RNAs were extracted using TRIzol reagent (Invitrogen) and then reversely transcribed using PrimeScript RT reagent Kit (TaKaRa, Dalian, China). Real-time PCR was performed using the ABI Prism 7300 sequence detection system (Applied Biosystems, Foster City, CA). The reaction mixture (20 μL) contained 10 ng cDNA template, 200 mM each of sense and antisense primers, and 10 μL 2 × SYBR-Green PCR Mix (TaKaRa). Real-time PCR was performed in duplicate for each sample, and the specificity of PCR product was estimated using the dissociation curve. β-actin was detected as the internal control.

Bioinformatics Analysis

To investigate the potential role of the IncRNAs–coexpressed mRNAs, these mRNAs were input into the Database for Annotation, Visualization, and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov) for annotation and functional analysis, including gene set enrichment analysis and mapping gene sets to the KEGG pathway.12 The TRANSFAC database was used to predict the transcription factor binding sites (TFBS) in the sequences of MALAT1 (http://www.gene-regulation.com).13 The catRAPID algorithm was used to predict the potential interacting proteins of MALAT1 (http://service.tartaglialab.com).14

IncRNA/mRNA Coexpression Network

To construct the IncRNA/mRNA coexpression network, we calculated the Pearson correlation coefficient and R value to evaluate IncRNA-mRNA correlation.15 The network construction procedures included the following: (1) preprocess data: the same mRNAs with different transcripts taking the median value represent the gene expression values, without special treatment of IncRNA expression value; (2) screen data: remove the subset of data according to the lists showing the differential expression of IncRNA and mRNA; (3) calculate the Pearson correlation coefficient and use R value to calculate the correlation coefficient between IncRNAs and mRNAs; and (4) screen by Pearson correlation coefficient: select the Pearson correlation coefficient greater than 0.99 as the meaningful value and draw the IncRNA/mRNA coexpression network by using the cytoscape program.

Clinical Sample Collection

The clinical study was approved by the ethics committees of Nanjing Medical University. The surgical specimens were handled in accordance with the Declaration of Helsinki. All patients gave informed consent before inclusion in this study. The FVMs were obtained from the patients who consecutively underwent pars plana vitrectomy as treatment of proliferative DR (PDR) caused by diabetes mellitus type 2 (study group) or who underwent pars plana vitrectomy as treatment of idiopathic macular holes or preretinal membranes (control group). Samples of aqueous humor were harvested from the eyes of participating patients who had PDR or nondiabetic ocular diseases. None of the patients with nondiabetic ocular diseases had diabetes mellitus.

Statistical Analysis

Data were presented as the mean ± SEM unless otherwise stated. Comparison between two groups was analyzed by using the two-tailed Student’s t-test or two-way ANOVA. Statistical significance was defined as P less than 0.05.

RESULTS

Induction of Diabetes in C57BL/6J Mice

Mice received intraperitoneal administration of STZ to induce diabetes. The control group received an injection of equal volume of citrate buffer. STZ treatment resulted in hyperglycemia and a progressive loss of body weight in STZ-treated mice (Table 1). Moreover, we compared ERG signaling in the
Aberrant Expression of IncRNAs in Early DR

| Table 1. General Physiological Parameters in Diabetic and Nondiabetic Mice |
|-----------------|-----------------|
|                  | Nondiabetic,    | Diabetic,          |
|                  | \( n = 10 \)    | \( n = 10 \)       |
| 2 wk after diabetic |                 |                    |
| Body weight, g    | 28.1 ± 4.5      | 27.8 ± 3.1         |
| Glucose, mg/dL    | 115 ± 6         | 278 ± 36*          |
| 4 wk after diabetic |                 |                    |
| Body weight, g    | 31.5 ± 5.2      | 29.7 ± 4.2*        |
| Glucose, mg/dL    | 110 ± 8         | 297 ± 44*          |
| 8 wk after diabetic |                 |                    |
| Body weight, g    | 37.6 ± 5.8      | 31.7 ± 2.9*        |
| Glucose, mg/dL    | 105 ± 4         | 515 ± 39*          |

* Significant differences between the nondiabetic and diabetic groups.

retinas of diabetic and nondiabetic mice after 2 months of STZ injection. A-wave response was found to be virtually identical between diabetic and nondiabetic retinas (Fig. 1A). At all light intensities evaluated, the retinas of diabetic mice showed reduced B-wave and oscillatory potential amplitudes (Figs. 1B, 1C). These findings were similar to previous reports, indicating that deleterious change in ERG signals occurred in the diabetic animal model, beginning at 6 weeks after STZ injection or even earlier. Taken together, these results suggested that STZ treatment resulted in an obvious change in retinal function in the retina of mice at the early stage of diabetes.

Overview of IncRNA Microarray Analysis

To reveal the potential role of IncRNAs in early DR, we performed a microarray analysis of the retinal tissues from STZ-induced diabetic mice and age- and sex-matched controls after 2 months of diabetes. The OD260:OD280 ratios of total RNAs were approximately 2.1, and the OD260:OD230 ratios of total RNAs were more than 1.9, suggesting that these RNAs were sufficiently pure for microarray analysis. Subsequently, these RNAs were reversed into cDNA transcripts, and microarray hybridization was performed using Agilent Mouse Gene Expression Microarrays (product number G4852A). The box plot provided an overview of IncRNA microarray data, which displayed the differences between samples without making any assumptions of the underlying statistical distribution. After normalization, the distribution of log 2 ratios between the nondiabetic and diabetic groups is shown in Figure 2A. Scatter plot provided an overall indication of sample similarity between individual transcripts. As shown in Figure 2B, the biological replicates exhibited similar transcript levels (nondiabetic versus nondiabetic, diabetic versus diabetic), whereas there was a significant IncRNA expression difference between nondiabetic and diabetic groups. To gain a systematic comparison of IncRNA expression between diabetic and nondiabetic retinas, we also used hierarchical clustering analysis to arrange samples into groups based on their expression levels. The diabetic samples were clustered together on the same branch, whereas the nondiabetic samples were clustered on the other branch (Fig. 2C).Taken together, these results suggested that this microarray analysis was completed with high quality. From an overall perspective, there was a significant difference of IncRNA expression between nondiabetic and diabetic groups.

Figure 1. ERG levels in the retinas of diabetic and nondiabetic mice. Mean ERG amplitudes for the retinas of diabetic and nondiabetic mice (\( n = 10 \) for each group) are shown. The top (A) shows the A-wave. The middle (B) represents the B-wave. The bottom (C) shows the amplitudes for the OPs.

Differential Expressed IncRNAs Between Nondiabetic and Diabetic Retinas

The microarray data were filtered by using the volcano plot to illustrate the differentially expressed IncRNAs between nondiabetic and diabetic retinas (Fig. 3A). We set a threshold as fold-change greater than 2.0, and identified 303 differentially expressed IncRNAs, including 214 downregulated IncRNAs and 89 upregulated IncRNAs (diabetic versus nondiabetic; Supplementary Table S1). To verify the results of microarray data, we performed real-time PCR assays to detect the expression differences of the top 10 upregulated and 10 downregulated IncRNAs between diabetic and nondiabetic retinas. We found that 9 of the 10 upregulated IncRNAs were verified to be significantly increased in diabetic retinas, whereas 8 of the 10 downregulated IncRNAs were verified to be decreased in diabetic retinas (Table 2).

We found that these differentially expressed IncRNAs showed different lengths ranging from 217 bp to 33,5 kb and were transcribed from the sense and antisense directions. Further, these differentially expressed IncRNAs were distributed in nearly all of the mouse chromosomes (Fig. 3B). In general, a number of IncRNAs showed “clusters of transcription” with multiple transcripts originating from relatively short segments of the genome. On mouse chromosome 1, two IncRNA clusters were found, which were respectively transcribed from a 138,499-bp (2437 bp, 10,001 bp, and 11,951 bp) or a 182,685-kb chromosomal region (3345 bp, 22,601 bp [forward (F)], and 22,601 bp [reverse (R)]); on mouse chromosome 2, three IncRNAs (18,426 bp, 634 bp, 4223 bp,
and 2456 bp) were transcribed from a 176,134-kb chromosomal region; on mouse chromosome 3, three lncRNAs (580 bp, 372 bp, and 10,551 bp) were transcribed from a 846,09-kb chromosomal region; on mouse chromosome 7, five lncRNAs (7276 bp, 15,076 bp, 1708 bp, 60,751 bp, and 7166 bp) were transcribed from a 134,369-kb chromosomal region; and on mouse chromosome 8, three lncRNAs (5876 bp, 6202 bp [F], and 6202 bp [R]) were transcribed from a 108,153-kb chromosomal region.

Construction of lncRNA/mRNA Coexpression Network

Although accumulating studies have attempted to reveal the functional significance of lncRNAs, the biological roles of most lncRNAs are still unknown. Biological processes and cellular regulation networks are very complex, involving the interactions of various molecules, such as proteins, RNAs, and DNAs.

The microarray data not only provided the information of lncRNA expression, but also provided mRNA expression information between nondiabetic and diabetic retinas. We thus constructed an lncRNA/mRNA coexpression network, and drew the regulatory network using the cytoscape program. The coexpression network was composed of 15 lncRNAs and 74 coexpressed mRNAs (Fig. 4). The network indicated that one mRNA could correlate with a great number of target lncRNAs and so were the lncRNAs, implying the inter-regulation of lncRNAs and mRNAs occurred in the early stage of DR.

Gene Enrichment and Pathway Analysis of lncRNAs–Coexpressed mRNAs

Gene enrichment analysis was performed to determine the gene and gene product enrichment in biological processes, cellular components, and molecular functions. We found that the highest enriched GOs targeted by lncRNAs–coexpressed mRNAs were cell response to stress (ontology: biological process), integral to membrane (ontology: cellular component), and structural molecule activity (ontology: molecular function; Fig. 5A). KEGG pathway analysis indicated that the lncRNAs–coexpressed mRNAs were involved in the regulation of axon guidance, MAPK signaling pathway, complement and coagulation cascades, chemokine signaling pathway, and pyruvate metabolism. One of these pathways, the gene category “axon guidance” is shown in Figure 5B, which has
been reported to be involved in the pathological process of ocular neurodegeneration.\textsuperscript{19,20}

Bioinformatics Analysis of MALAT1

MALAT1 is one of the highly conserved lncRNAs in mammals. It is located on chromosome 11q13, and is known to be misregulated in several solid tumors and associated with cancer metastasis and recurrence.\textsuperscript{8} In the results of our microarray data, we found that an orthology of MALAT1, linRNA chr19: 53624826-53625580, was aberrantly expressed in diabetic retinas (Fig. 6A). Transcription factors were recognized as important components of signaling cascades controlling all types of normal cellular processes as well as response to external stimulus.\textsuperscript{13} Here, we used the TRANSFAC program to predict the TFBS in the sequences of MALAT1. The result indicated that MALAT1 could combine with nuclear factor-kB (NF-kB) motif as the cis-acting element, respectively (Fig. 6B).

We also conducted catRAPID analysis to predict the potential interacting protein of MALAT1. We found a strong interaction between MALAT1 and CPNL1 (Fig. 6C).

**Clinical Relevance**

To reveal the potential role of MALAT1 in the pathogenesis of ocular diseases, we first treated RF/6A cells with high levels of glucose (50 mM) to mimic the diabetic condition in vitro, and then detected the expression pattern of MALAT1. We found that high glucose resulted in approximately 40% increase in MALAT1 levels for the first 24 hours, and an approximately 2.2- and 4.1-fold increase in MALAT1 levels after 36- and 48-hour high glucose treatment. By contrast, the expression of MALAT1 was stably expressed during the experimental period in the normal glucose (5 mM) group and mannitol-treated group (Fig. 7A).

To reveal the clinical relevance of MALAT1 misregulation, we conducted real-time PCR to examine MALAT1 levels in the aqueous humor and in the FVMs of patients with PDR. The clinical characteristics of the patients are shown in Table 3. Results of quantitative RT-PCR showed that MALAT1 expression was significantly higher in the FVMs of the nine patients with PDR than in the membranes of the six patients with idiopathic epiretinal membranes (Fig. 7B). We also found that MALAT1 level was significantly higher in the aqueous humor of PDR patients than that of nondiabetic patients (Fig. 7C). Taken together, these results support the hypothesis that MALAT1 misregulation is a potential molecular mechanism of DR pathogenesis.

**DISCUSSION**

In recent years, the concept of the functional genome has been rewritten to encompass a multitude of newly discovered noncoding RNA transcripts. The functional significance of
Figure 4. The lncRNA/mRNA coexpression network constructed using the cytoscape program. (A) The lncRNAs and mRNAs with Pearson correlation coefficients not less than 0.99 were selected to draw the regulatory network by using the cytoscape program. (B) Real-time PCR experiments were conducted to verify the expression associations between lncRNAs and mRNAs.
lncRNAs has long been recognized. However, the change in the abundance and scale of lncRNA expression in ocular diseases is just beginning to come to light. DR is a result of multiple pathogenetic processes caused by hyperglycemia and abnormalities of insulin-signaling pathways, leading to retinal microvascular defects and neuroretinal dysfunction and degeneration. Although significant progress has been made, the molecular mechanisms underlying DR pathogenesis are still not fully understood. For this reason, charting the transcriptional landscape of lncRNAs is a key step in understanding the significance of lncRNAs in DR. Here, we detected lncRNA profiling in a murine model of DR by using microarray analysis. We identified that 303 lncRNAs were aberrantly expressed at the early stage of DR. Moreover, we found that MALAT1, a conserved lncRNA, was significantly upregulated in the RF/6A cell model of hyperglycemia, in the aqueous humor, and the FVMs of DR patients, implying its application as a biomarker for the prognosis and diagnosis of DR.

To date, several lncRNAs have been implicated in eye development, including Vax2os1, RNCR2, Six3OS, and Tug1. Vax2os1 controls the cell cycle progression of photoreceptor progenitors in the mouse retina. RNCR2, Six3OS, and Tug1 play a critical role in regulating retinal cell fate specification. Islet-cell dysfunction is central to the pathophysiology of type 2 diabetes. Type 2 diabetes would manifest when the β-cell fails to secrete sufficient amounts of insulin to maintain normoglycemia and undergoes apoptosis. Human β-cell transcriptome analysis uncovers that lncRNAs are tissue specific, dynamically regulated, and abnormally expressed in type 2 diabetes. In addition, GWAS study indicates that antisense noncoding RNA in the INK4 locus (ANRIL) is significantly associated with increased susceptibility to type 2 diabetes. Therefore, it is no
surprise that abnormal lncRNA expression may be relevant to the molecular etiology of DR. The identification of dysregulated lncRNAs may also open a new framework to study the pathophysiology of diabetes and diabetic complications.

Compared with the protein-coding sequences, most lncRNAs are poorly conserved throughout vertebrates. It is difficult to predict the functions of lncRNAs based on their nucleotide sequences. To reveal the functional significance of lncRNAs in DR, we constructed the lncRNA/mRNA coexpression network based on the correlation analysis. lncRNAs-coexpressed mRNAs are targeted to "cellular response to stress" (ontology: biological process), "integral to membrane" (ontology: cellular component), and "structural molecule activity" (ontology: molecular function). As shown in

**Figure 6.** Bioinformatics analysis of MALAT1. (A) The chromosome location of MALAT1 is shown in the mouse genome. (B) Transcription factor binding site prediction indicated that NF-κB was the cis-acting element of MALAT1. (C) catRAPID analysis indicated a strong interaction between CPNL1 and MALAT1.
Aberrant Expression of lncRNAs in Early DR

In a recent study, Mustafi et al. identified only 18 conserved lncRNAs from 3133 mouse lncRNAs—coexpressed lncRNAs–coexpressed lncRNAs. These signaling pathways are tightly associated with the pathological processes, such as neovascularization, inflammation, and immunology. Suggesting the lncRNA-mediated network plays a wide role in the pathogenesis of DR.

Expression pattern of MALAT1 in RF/6A cells, the aqueous humor, and FVMs of DR patients. (A) RF/6A cells were incubated in media containing 5 mM glucose, 50 mM glucose, or 50 mM mannitol for 24 hours, 36 hours, and 48 hours. The group treated with 5 mM glucose was taken as the control group, and the group treated with mannitol was taken as the osmolar control. The levels of MALAT1 were determined by real-time PCR. (B) The FVMs were collected from the eyes of patients with PDR, or with macular holes or preretinal membranes. Real-time PCR was conducted to detect the level of MALAT1 expression. (C) Samples of aqueous humor were harvested from the eyes of patients who had PDR or nondiabetic ocular diseases. None of the patients with nondiabetic ocular diseases had diabetes mellitus. The levels of MALAT1 were determined by real-time PCR. The data of each group are expressed as the relative change compared with the control group. The asterisk indicates the significant difference compared with the control group.

Figure 5A, the biological processes, such as epithelium and tube development, may be involved in the regulation of retinal vascular leakage. The biological processes, including tube morphogenesis, epithelium development, and branching morphogenesis of a tube, may be involved in the process of pathological neovascularization. IncRNAs–coexpressed mRNAs are also targeted to several signaling pathways, including axon guidance, MAPK signaling pathway, complement and coagulation cascades, chemokine signaling pathway, and pyruvate metabolism. These signaling pathways are tightly associated with the pathological processes, such as neurodegeneration, neovascularization, inflammation, and immunology.

Changes in aqueous humor protein content have been detected in other body fluids, suggesting the lncRNA-mediated network plays a wide role in the pathogenesis of DR. Of them, “axon guidance” is essential for the establishment of proper neuronal connections during development, which gains on the top count score during KEGG analysis. This result implies that neurodegeneration is an early event in the pathogenesis of DR. The finding is in accordance with previous clinical evidence from ERG, contrast sensitivity, perimetric, and color vision studies, which suggests that neuronal changes may occur before clinically detectable microvesselopathy.

Compared with the protein-coding sequences, lncRNA sequences evolve very rapidly. In a recent study, Mustafi et al. identified only 18 conserved lncRNAs from 3133 mouse lncRNAs, which is similar to other studies that showed only a small minority of lncRNAs in the mouse or human have transcribed orthologous sequences across different species. By contrast, the highly conserved lncRNAs would play a critical and conserved role across different species. MALAT1 is a highly conserved lncRNA across different mammalian species. MALAT1 has been reported to be significantly upregulated in several solid tumors and is linked to cancer metastasis and recurrence. In this study, we found that MALAT1 is significantly upregulated in the retinas of diabetic mice, in the RF/6A cell model of hyperglycemia, in the aqueous humor samples, and FVMs of diabetic patients. Thus, we provide the evidence from cell line, animal model, and clinical samples, which indicate that MALAT1 dysregulation may become the molecular etiology of DR occurrence.

Regular ophthalmological examinations, timely laser therapy depending on the stage of the disease, and close interdisciplinary cooperation are essential to prevent loss of vision in DR. However, the prognosis for DR patients is still poor. Aqueous humor is an important intraocular fluid responsible for supplying nutrients to and the removal of metabolic wastes from the avascular tissues of the eye. Changes in aqueous humor protein content have been associated with potentially blinding diseases, such as primary congenital glaucoma, myopia, and Fuchs endothelial corneal dystrophy. Based on the fact that lncRNAs have been detected in other body fluids, we speculated that they may also be present in aqueous humor. Importantly, we identified that MALAT1 expression is significantly upregulated in the aqueous humor of DR patients. The change in MALAT1 level may affect the physiological function of ocular tissue, which may become an indicator in the early stage of DR.

<table>
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<th>Clinical Parameters</th>
<th>FVMs</th>
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<tr>
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<tr>
<td>Glycosylated hemoglobin</td>
<td>7.2 ± 0.85</td>
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MALAT1 may be developed as a biomarker for diagnosing or making a prognosis of DR based on the MALAT1 levels.

FVMs form on the surface of the neuroretina as a sequela to PDR. FVMs are characterized by the migration and proliferation of various types of cells (e.g., retinal glial cells, fibroblasts, macrophages/monocytes, hyalocytes, laminocytes, and vascular endothelial cells). The BFGE VEGF TNF-α, angioptiogenin-2, hepatocyte growth factor, monocyte chemotactic protein-1, IL-8, NF-xB, and activator protein-1 have been detected in FVMs collected from PDR patients. These observations support the concept that a complex local milieu, rather than a single or a few growth factors, influences the generation of FVMs. Here, MALAT1 is significantly upregulated in the FVMs of patients with PDR. MALAT1 is a critical regulator of cell motility, cell cycle progression, and cell proliferation. Thus, it is no surprise that MALAT1 dysregulation could affect the migration and proliferation of FVM-related cells, which affect the DR pathogenesis.

DR is one of the most important diabetic complications. Genetic factors, environmental factors, and the complex gene/environment interactions may be implicated in the pathogenesis of DR. Here, we present the detailed data designed to serve as a resource for elucidating lncRNA-mediated DR pathogenesis. Further, we found that MALAT1, a conserved lncRNA, may become a potential biomarker for the prognosis and diagnosis of DR. More studies are required to investigate the correlations between lncRNA change and DR development at different stages. Moreover, in vivo and ex vivo studies should be conducted to elucidate the molecular mechanisms of lncRNA-mediated DR occurrence and estimate their potential for the prognosis, diagnosis, and treatment of DR.

Acknowledgments

Supported by the National Natural Science Foundation Grants 81300241 (BY), 81271028 (JY), and 81371055 (QJ); the National Clinical Key Construction Project Grant (2012) 649 (QJ); and the Medical Science and Technology Development Project Fund of Clinical Key Construction Project Grant (2012) 649 (QJ); and the Medical Science and Technology Development Project Fund of Nanjing Grants ZKX12047 (QJ), YKK13227 (BY), and YKK12208 (JY).

Disclosure: B. Yan, None; Z.-F. Tao, None; X.-M. Li, None; H. Zhang, None; J. Yao, None; Q. Jiang, None

References


