Long Noncoding RNAs in Diabetic Retinopathy
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With the advent of next generation sequencing technologies it became evident that the majority of the human genome is transcribed, whereas only 1.5% to 2% of the genome encodes proteins. The remaining transcripts are referred to as noncoding RNAs. With respect to microvascular dysfunction, functional roles of noncoding RNAs are in particular evident for microRNAs (miRNAs, miRs), which belong to the group of small noncoding RNAs (<200 nt). In contrast, the contribution of long noncoding RNAs (lncRNAs; >200 nt) is not conclusively defined. Even though the particular functions of lncRNAs seem to be diverse, their mechanisms of action can be grouped into 3 main themes. Hence, lncRNAs can (1) function as decoys for proteins or RNAs, (2) serve as scaffolds for higher order complexes, or (3) act as molecular guides to ensure the proper localization of their binding partners. Taking these central functions into account, which finally encompass the fields of transcriptional and post-transcriptional regulation, as well as subcellular dynamics, dysregulation of lncRNA expression is often associated with complex human diseases. In the field of vascular biology, the importance of lncRNAs in cellular homeostasis was recently uncovered by the finding that the lncRNA MALAT1 was identified to be crucial for the angiogenic response of endothelial cells as well as for vascularization in vivo.

In this issue of Circulation Research, Yan et al. revealed a regulatory role of the lncRNA myocardial infarction–associate transcript (MIAT) in diabetes mellitus–induced microvascular dysfunction. MIAT (which is also known as RNRCC2, AK028326, or Gomafu) was first identified as susceptible locus for myocardial infarction and was reported to be highly expressed in retinal precursor cells. Manipulation of MIAT triggers pleiotropic effects on brain development, which are at least in part mediated by aberrant splicing of Wnt7b. Yan et al. now show that MIAT is strongly upregulated in the retinas of diabetic rats and patients. Consistently, high glucose conditions trigger upregulation of MIAT in vitro. In turn, interference with MIAT expression was found to improve functional under diabetic conditions along with beneficial effects for retinal vessel impairment. In particular, MIAT knockdown in vivo was shown to be accompanied by reduced vascular leakage and by counteracting the diabetes mellitus–induced upregulation of proinflammatory proteins, thereby alleviating retinal vessel impairment (Figure). The mechanisms underlying these impressive therapeutic benefits are not entirely clear. Because endothelial cells are considered as primary cellular targets in diabetes mellitus–induced vascular disease, the authors investigated the role of MIAT in cultured endothelial cells. siRNAs directed against MIAT significantly decreased endothelial inflammatory responses, which is conceivable with the therapeutic benefits seen in vivo. However, MIAT knockdown resulted in elevated apoptosis under basal conditions and reactive oxygen species stimulation, a finding which contradicts their observation that MIAT silencing in vivo increases activation of antiapoptotic kinase Akt and reduces the number of TUNEL-positive cells. The discrepancy between the in vitro and in vivo study may have multiple reasons; one may speculate that the three-dimen- sional in vivo context influences the response of endothelial cells with respect to MIAT silencing. Moreover, MIAT may have functions in other cell types which contribute to the in vivo phenotype. For instance, MIAT is also expressed in retinal precursor cells and inflammatory cells and silencing by shRNA is not specific for endothelial cells. Therefore, MIAT silencing may affect the function of other cell types, which may contribute indirectly (eg, by changing the paracrine environment) to the normalization of endothelial functions and to improved endothelial cell survival under diabetic conditions. Although the mechanisms underlying the in vivo function of MIAT deserve further investigation, this study is the first to address the role of lncRNAs in diabetic retinopathy and shows an impressive therapeutic benefit. To date, little is known about lncRNA functions under diabetic conditions: MALAT1 was recently shown to control endothelial cell functions not only in ischemia models but also under diabetic conditions. Likewise, some lncRNAs such as H19 were implicated in the control of metabolic alterations induced by diabetes mellitus.

Yan et al. studied the molecular mechanisms that regulate MIAT expression and mediate its functions in endothelial cells. The authors showed that the microRNA miR-150-5p targets MIAT in endothelial cells in vitro. Inhibition of miR-150-5p in the eyes increased MIAT levels and vice versa, miR-150 mimics reduce basal and diabetes mellitus–induced upregulated MIAT expression. Because miR-150-5p binds to and interferes with MIAT expression, a putative role of MIAT as competing endogenous RNA was analyzed. Competing endogenous RNAs are supposed to exhibit gene regulatory properties by competing with messenger RNAs (mRNA) for a shared set of microRNAs, thereby regulating the translation of their antagonist. Several studies support the concept that miRNAs may act as
sponges for microRNAs. However, the competing endogenous RNA hypothesis has been discussed controversially because it is difficult to imagine how changes in the expression of an individual miRNA, which contributes only to a minor fraction of the overall miRNA targets, could influence enough miRNA molecules to affect gene expression. Denzler et al.\(^4\) recently tested this hypothesis and showed that overexpression of miRNA can affect miRNA target derepression only if \(\approx 6000–7000\) competing miRNA-binding sequences were overexpressed. Whether the same stoichiometry is required, if noncoding RNAs (instead of mRNAs) are used to scavenge microRNAs needs to be determined. Several publications showed that noncoding RNAs such as circRNAs, which comprise \(>60\) miRNA binding sites, or long noncoding RNAs in the cardiovascular system can act as sponges for miRNAs.\(^5,6\) Yan et al.\(^6\) specifically addressed whether MIAT acts as a sponge for miR-150. Two prerequisites have to be fulfilled to ensure that a given lncRNA can act as microRNA sponge: (1) The lncRNA and the corresponding miRNAs have to be expressed in the same cellular compartment, and (2) a reasonable stoichiometric ratio of the lncRNA and the given miRNA. In this context, the lncRNA must have multiple miRNA binding sites or must be expressed at levels that are sufficient to bind a biologically meaningful number of miRNA molecules. The latter point is particularly important because most lncRNAs are expressed at lower levels compared with miRNAs. In this study, Yan et al.\(^6\) carefully controlled the localization of MIAT and showed that MIAT and miR-150 are both nuclear localized and thus may bind each other. Although microRNA-mediated silencing occurs predominantly in the cytoplasm, Argonaute-2 has been previously reported in the nucleus.\(^7\) However, as mentioned above, the stoichiometry needs to be carefully considered: MIAT does not belong to the highly expressed lncRNAs in endothelial cells under basal conditions (1-10 reads per kilobase of transcript per million–mapped reads \(<0.05\))\(^5\) and has only 1 miR-150–binding site. Additionally, the expression of miR-150–5p is low in endothelial cells (1-10 reads per kilobase of transcript per million mapped reads compared with values of 6000–7000 for prototypical endothelial miRNAs, eg, miR-126-5p). Although absolute reads cannot be directly compared between mRNA/lncRNA and microRNA sequencing, the low expression of miR-150–5p suggests that in principle this microRNA could be scavenged by an lncRNA. Indeed, overexpression studies with miR-150 and MIAT clearly support a regulation of the miR-150 target vascular endothelial growth factor. However, the putative sponge function of MIAT has only been documented after overexpression of MIAT, which may have resulted in artificially high levels of the lncRNA. Given the epigenetic functions of lncRNAs and the established role of MIAT in regulating miRNA splicing,\(^2\) alternative explanations for a MIAT-dependent regulation of vascular endothelial growth factor expression may have to be considered as well. An interesting experiment would have been to determine whether diabetes mellitus–induced MIAT upregulation is sufficient to derepress miR-150 target genes and whether MIAT-dependent regulation of vascular endothelial growth factor is causally mediated by miR-150.

In summary, the study by Yan et al.\(^6\) provides important and novel insights into the regulation and function of lncRNAs in the vasculature. Given that the lncRNA field is still in its infancy, many questions are arising that need to be addressed in the future. The therapeutic benefits seen after shRNA-mediated silencing opens up novel therapeutic opportunities to target cardiovascular diseases. The eye is easily accessible, however, for other indications, antisense approaches, which do not require vector-based overexpression, might be advantageous. The development of safe and efficient tools to modulate lncRNA functions, as it has been done for microRNAs,\(^8\) may be helpful to develop therapies for other cardiovascular diseases.

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**Disclosures**

We submitted a patent application on lncRNAs in cardiovascular diseases.

**References**


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