Mugdha V. Joglekar,[1] Andrzej S. Januszewski,[2] Alicia J. Jenkins,[2] and Anandwardhan A. Hardikar[1]

Circulating microRNA Biomarkers of Diabetic Retinopathy

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COMMENTARY

The incidence of diabetes is increasing globally, and in spite of modern treatments for glucose, blood pressure, and lipids, the vascular and neurologic complications of diabetes still occur at unacceptably high rates. The personal and economic burdens are enormous. Diabetes is a common cause for vision loss (diabetic retinopathy [DR]), renal impairment (nephropathy), peripheral and autonomic neuropathy and accelerated atherosclerosis leading to cardiovascular disease, cerebrovascular and peripheral vascular disease, and premature death (1). People with diabetes and with early renal damage are at particularly high risk of DR, renal failure, premature cardiovascular disease, and death. Moreover, people with DR are at increased risk of other micro- and macrovascular complications of diabetes (2). This may relate to common risk factors and mechanisms, such as damage to the vasculature, a chronic and clinically silent process. Vascular endothelial cells are the interface between blood and surrounding tissues, with key roles in regulation of inflammation, thrombosis, and angiogenesis and therefore a popular choice as a model for assessing endothelial damage and metabolic stress (3,4).

Vision loss due to DR is often preventable. However, for many people with diabetes it is often difficult to predict the clinical course of disease. Furthermore, not all people take or respond well to proven primary and secondary treatments for DR, including risk factor control, renin-angiotensin system drugs, fenofibrate, and late-stage treatments, such as ocular laser, corticosteroids, and anti–vascular endothelial growth factor agents. This highlights the importance of improving our understanding of the pathogenesis of DR and developing newer treatments and the necessity for clinical laboratory–based screening and monitoring tests (or panels thereof) that are highly sensitive and specific, widely available, and cost-effective for this potentially blinding condition. The field of oncology uses molecular markers in (clinical and basic) research and in clinical practice, but the use of such tools in diabetes is lacking. In this issue of *Diabetes*, the article by Zampetaki et al. (3) is an important advance in the field of microRNA (miRNA) biomarkers that has implications in the areas of disease (retinopathy) pathogenesis, prognostication, monitoring, and therapeutics.

miRNAs are a group of small (~22 nucleotide) ubiquitously expressed RNA molecules that do not code for any protein but act posttranscriptionally to modulate expression of target genes via inhibition of protein expression by interfering with the translation and/or stability of mRNA. Cells release miRNAs into the circulation, where they have a long life span (approximately ≥2 weeks). Their stability in plasma/serum/urine on freeze-thawing, efficient recovery, and availability of quantitative detection methods enhances their use as *a biomarker as well as a potential mediator of physiological and pathological processes*. There is a paucity of miRNA clinical studies in DR, hence the importance of the study by Zampetaki et al. (3).

Zampetaki et al. (3) assessed 300 samples from two DR-related randomized, double-blind, parallel-design, and placebo-controlled clinical trials (PROTECT-1 and PREVENT-1). The authors assessed 155 baseline or DR progressors and 145 control samples (selected from 3,326 study participants) for a panel of 29 candidate miRNAs that were based on previous studies related to diabetes and myocardial infarction. They identified miR-27b and miR-320a as being significantly and independently associated with high DR risk. Complementing this analysis, they also elucidated the potential mechanism using cultured human endothelial cells and identified antiangiogenic thrombospondin-1 as a common target of these two miRNAs.

This study uses human clinical samples from existent DR trials, which is time-saving and cost-effective, and assays mature miRNAs on a robust gold-standard real-time PCR platform (5). The authors acknowledge the study strengths and most of its limitations. A weakness is the use of a

[1]Diabetes and Islet Biology Group, NHMRC Clinical Trials Centre, University of Sydney, Sydney, New South Wales, Australia
[2]Vascular Biology and Biomarker Laboratory, NHMRC Clinical Trials Centre, University of Sydney, Sydney, New South Wales, Australia
Corresponding author: Anandwardhan A. Hardikar, anand.hardikar@ctc.usyd.edu.au.

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candidate miRNA approach rather than an unprejudiced discovery (small RNA-sequencing) and validation approach. This is increasingly important as a single miRNA targets multiple (even hundreds) protein-coding gene transcripts, and candidate miRNAs important in diabetes or myocardial infarction may not be the best signature for DR. With regard to the cell culture studies, evaluation of other retinal cell types, cocultured retinal cells, and oscillating glucose concentrations in culture media, as occurs in vivo, would also have been of interest.

With regard to the statistical analysis, two complementary approaches, classical logistic regression (LR) and L1-penalized logistic regression (PLR), were used. Using LR for selecting markers from gene expression arrays can be prone to problems with multicollinearity and overfitting, but the results of LR are easy to present and interpret. PLR performs good model selection by shrinking the components of β to zero, effectively deleting these coefficients. For the PROTECT-1 arm, this method might have overestimated λ and contributed to the low DR predictive power (see Supplementary Fig. 5*B* in ref. 3). An alternate approach may be the use of Akaike information criterion to choose the λ value for PLR.

So far, there are few studies aimed at understanding the dysregulated miRNAs in DR, which we have summarized in Fig. 1. The majority use retinal tissues of various animal models or endothelial cells exposed to high glucose conditions in vitro (4,6–15). Very few studies have used clinical samples (3,5,16–18) or complementary clinical and basic science approaches, as Zampetaki et al. (3) did. We hope that this important report is followed by more clinical studies in which miRNAs are evaluated in larger study cohorts, across different ethnic groups, in different types of diabetes, and in a wide age range as well as different stages of DR. The effects of clinical, pharmacologic, and diabetes device–related interventions on miRNA profiles and their relationships to clinical end points are of great interest. It would also be of interest to see small RNA-sequencing analyses from such trials and to follow up these (or other identified) miRNAs in longitudinal studies to determine their predictive value for DR progression and responses to treatments. As miRNAs themselves may be therapeutic targets or even therapeutic agents (as

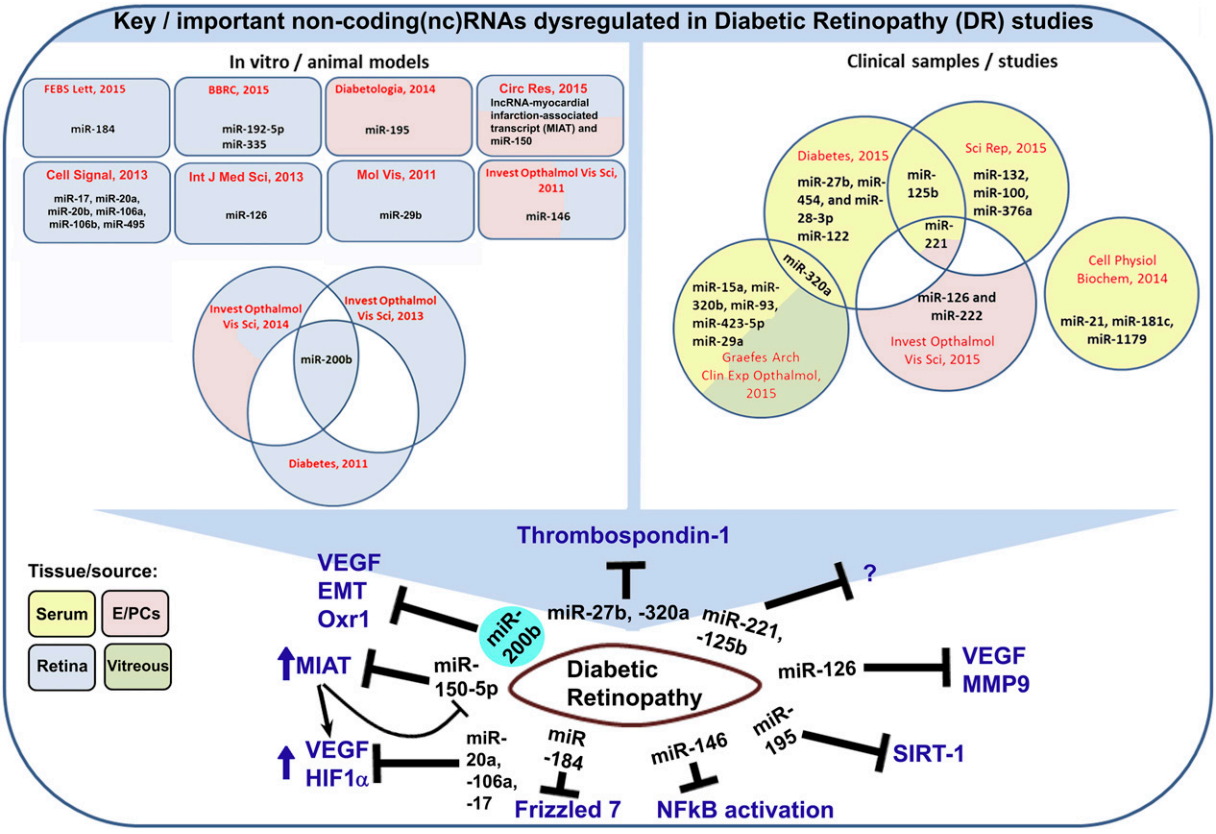

Figure 1—Some of the important miRNAs from various studies attempting to identify dysregulated miRNAs in DR. The left panel shows miRNAs from in vitro experiments or animal models, and the right panel shows miRNAs reported from clinical studies. Commonly identified miRNAs are represented by intersecting circles. The background colors represent tissue sources for miRNA assessment. The lower panel shows miRNAs and their targets reported to be altered in DR. miR-200b (cyan-colored background) is currently being tested for therapeutic potential in DR. The predictive potential of these (or other) miRNAs will be revealed after careful assessment and validation in multiple clinical trials. EMT, endothelial to mesenchymal transition; E/PCs, endothelial or endothelial progenitor cells; HIF1α, hypoxia inducible factor-1α; MIAT, myocardial infarction–associated transcript; MMP9, matrix metalloproteinase-9, NFkB, nuclear factor-κB; Oxr1, oxidation resistance-1; SIRT1, sirtuin-1; VEGF, vascular endothelial growth factor.

anti-miRNAs) (15), further studies will help in identifying and assessing their therapeutic potential for the treatment of retinopathy in individuals with diabetes.

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