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Targeting miR-200c to Ameliorate Diabetes-Induced Endothelial Dysfunction

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COMMENTARY

Endothelial dysfunction is one of the earliest manifestations of diabetes that can lead to numerous debilitating micro- and macrovascular complications (1,2). Identifying factors that mediate or augment endothelial dysfunction in diabetes can lead to the development of much-needed therapies that target early stages of various vascular complications and thereby prevent further progression. Chronic endothelial dysfunction includes impaired endothelium-dependent vasorelaxation (EDR), increased inflammation, and dysregulation of vascular remodeling (1–3). Inactivation or reduced bioavailability of gasotransmitter nitric oxide (NO), also known as EDR factor, is a key factor involved in the development of vascular diseases such as hypertension and atherosclerosis (1,4), which are accelerated under diabetic conditions. NO is produced by endothelial NO synthase (eNOS), and several studies have demonstrated increased oxidative stress, eNOS uncoupling, and reduced NO bioavailability under diabetic conditions (1–4). In addition to NO, endothelial cells (ECs) also produce several prostanoids with vasodilator and vasoconstrictor activities (5) mainly via the actions of the cyclooygenase enzymes COX-1 and COX-2. A fine balance between the levels of these prostanoids contributes to normal EDR. COX-2 produces both prostacyclin (PGI_2), which reduces platelet aggregation, and prostaglandin E_2 (PGE_2), which can reduce EDR. In diabetes, high glucose (HG)-induced oxidative stress and reactive oxygen species (ROS) increase the expression of COX-2 and prostaglandin H_2 (PGH_2), a common precursor of prostaglandins. However, HG-induced ROS inhibit further metabolism of PGH_2 into the vasodilator PGI_2 via inhibition of PGI_2 synthase (6), but platelet thromboxane is not affected. This shifts the balance toward thrombosis, which could be a reason for adverse cardiovascular events associated with COX-2 inhibitors (7). The article by Zhang et al. (8) in this issue of *Diabetes* sheds new light on the mechanisms involved in COX-2 upregulation and impairment of EDR in the diabetic endothelium.

microRNAs (miRNAs) are endogenously produced short noncoding RNAs that modulate gene expression by triggering posttranscriptional gene silencing. They function by binding to the 3′ untranslated regions of specific target mRNA sequences, leading to mRNA degradation or inhibition of translation (9). miRNAs have received considerable attention as potential biomarkers and therapeutic targets for cardiometabolic diseases and other diabetic vascular complications because they can modulate the expression of major regulators of endothelial and vascular smooth muscle cell (VSMC) functions related to vascular tone and inflammation (10–13).

Previous studies have shown that certain members of the miR-200 family are misregulated in diabetes and various diabetes complications including nephropathy, retinopathy, and vascular inflammation (14–17). The miR-200 family includes five members expressed from two polycistronic transcripts that encode the miR-141/miR-200a cluster and miR-200b/miR-200c/miR-429 cluster. Well-studied targets of miR-200 include the transcription regulators ZEB1 and ZEB2, which regulate genes involved in epithelial-to-mesenchymal transition (EMT), angiogenesis, fibrosis, and inflammation (15,17,18). In cancer, loss of miR-200 increases ZEB1/ZEB2 to promote EMT and metastasis (18). However, in diabetes, the regulation and actions of miR-200 vary depending on the cell and tissue type affected in the complications. Some members of the miR-200 family were found to be upregulated in renal mesangial cells treated with transforming growth factor-β1, in glomeruli of mouse models of diabetic nephropathy and in VSMCs derived from aortas of type 2 diabetic *db/db* mice. In parallel, the miR-200 targets ZEB1/ZEB2 were downregulated, which led to increases in the expression of fibrotic and inflammatory genes in mesangial cells and VSMCs, respectively (15,17). In contrast, some

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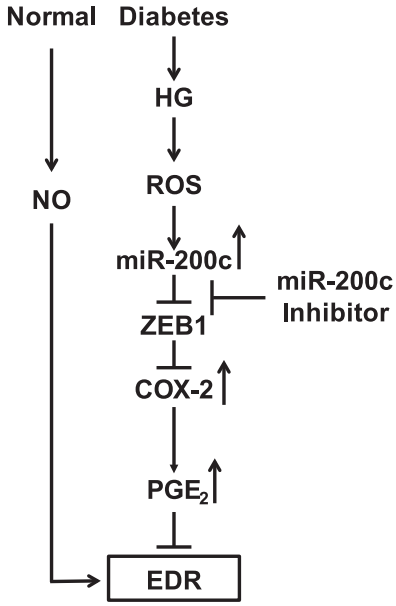

Figure 1—Schematic depicting the role of miR-200c in diabetes-induced EC dysfunction: EDR plays an important role in normal endothelial function. In diabetes, increased ROS leads to increases in miR-200c, which upregulates COX-2 by targeting transcription repressor ZEB1. Dysregulated production of COX-2–generated prostanoids increases levels of PGE_2, which inhibits EDR and promotes endothelial dysfunction implicated in diabetic vascular complications.

reports also showed decreases in miR-200a in renal tubular epithelial cells under diabetic conditions, which was also associated with fibrosis (19). In retinal ECs, miR-200b was again downregulated leading to increases in vascular endothelial growth factor and angiogenic factor involved in the pathogenesis of diabetic retinopathy (16). Thus miR-200 has been widely studied, but its role in EDR and diabetic macrovascular complications is not well known.

In the current issue, Zhang et al. (8) show that miR-200c is increased in aortas from type 2 diabetic *db/db* mice and renal arteries from humans with diabetes. In vitro experiments showed that HG upregulates miR-200c in an ROS-dependent mechanism in ECs. miR-200c overexpression ex vivo in nondiabetic aortas could inhibit EDR, and miR-200c inhibitors improved EDR in diabetic aortas. miR-200c levels inversely correlated with ZEB1 levels, and adenoviruses expressing ZEB1 improved EDR ex vivo in diabetic aortas. Furthermore, the authors elegantly showed that tail-vein injection of miR-200c inhibitor or ZEB1-expressing adenoviruses ameliorated impaired EDR in diabetic mice, confirming in vivo relevance. Mechanistic studies showed that miR-200c overexpression or ZEB1 inhibition could upregulate COX-2 expression, and this was associated with increases in vasoconstrictor prostaglandins. Notably, COX-2 knockout mice were resistant to miR-200c–induced impairment of EDR, clearly demonstrating the mediatory role of COX-2. These findings provide a new mechanistic understanding of diabetes-induced EC dysfunction via COX-2 upregulation due to miR-200c–mediated ZEB1 inhibition (Fig. 1). They also illustrate the potential of targeting miR-200c for reversing diabetes-induced EC dysfunction.

However, the authors did not examine other miR-200 members, unlike a previous study, which showed that miR-200b and miR-429 were also increased in aortas from *db/db* mice (15). The current study also did not examine the role of the miR-200c–ZEB1 axis in endothelial inflammation responses including expression of adhesion molecules and monocyte–EC binding. Such studies could further highlight the pathological role of miR-200c in inflammation evoked by diabetes-induced ROS in ECs. Interestingly, adenoviruses expressing miR-200c inhibitors restored EDR in *db/db* mice, a finding that could warrant further evaluation of the therapeutic benefit of miR-200c inhibitors in models of diabetic vascular complications like atherosclerosis. This approach might also help in reducing the prothrombotic side effects of COX-2 inhibition. However, much work is needed before this can be translated to the clinic. Owing to differences in the cell-type regulation of miR-200 under diabetic conditions, potential side effects of miR-200c inhibitor therapy should be evaluated. The authors also tested the functions of only one of the targets of miR-200c, ZEB1. As miRNAs can have multiple targets, the "off-target" effects of the anti-miR treatment, as well as specificity to the endothelium versus other sites, need to be systematically checked, as does the potential toxicity of long-term treatment regimens. Several improvements in miRNA targeting are emerging including the use of specific chemical modifications for anti-miRNA oligonucleotides (e.g., locked nucleic acids) that reduce toxicity and enhance bioavailability, in vivo stability, and tissue-specific delivery (20). The use of multifunctional nanoparticles to deliver therapeutic miRNAs to inflamed proatherogenic regions offers a promising approach for targeted delivery (21). The current study adds to the growing list of miRNAs that appear to play a role in diabetic vascular dysfunction. Further developments in the safety and efficacy of miRNA therapeutics in animal models might lead to better treatments for diabetic vascular complications in humans. Given the paucity of effective therapies for diabetic endothelial dysfunction, miR-200c could be an attractive target.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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