Diabetes mellitus impairs physiological angiogenesis, which may be manifested as nonhealing foot ulcers or refractory angina. Multiple molecular mechanisms have been proposed. Hyperglycemia induces the generation of reactive oxygen species that cause endothelial derangements, including the reduced synthesis of endothelium-derived nitric oxide (NO). The bioactivity of NO is critical for angiogenic processes such as the survival, proliferation, and migration of endothelial cells. The impairment in NO bioactivity may also explain in part the reduced expression of a major angiogenic cytokine, vascular endothelial growth factor (VEGF), in hyperglycemic states, because NO and VEGF have a reinforcing and reciprocal relationship. Glucose intolerance also reduces the number and function of bone marrow–derived endothelial progenitor cells, circulating cells that participate in the angiogenic response. In addition to generating reactive oxygen species, hyperglycemia may impair cytoprotective mechanisms against oxidative stress. In particular, the thioredoxins play a key role in angiogenic processes by maintaining endothelial redox homeostasis, with favorable effects on protein folding, activity of reductive and metabolic enzymes, energy utilization, and transcription factor activity. Emerging evidence indicates that hyperglycemia upsets this cytoprotective mechanism by increasing the expression of the endogenous inhibitor thioredoxin-interacting protein (TXNIP).

Is it possible that these and other disparate mechanisms for the impaired angiogenesis in diabetes mellitus have a common genomic basis? This question logically follows from the work of Caporali and colleagues in the current issue of *Circulation.* They have discovered a novel genomic mechanism for hyperglycemia-induced impairment of angiogenesis, ie, the increased expression of a specific microRNA (miRNA) that appears to orchestrate a pathophysiological response in diabetes mellitus.

miRNA and Genomic Regulation

Accumulating data indicate that noncoding RNA plays a critical role in genomic regulation. One of these noncoding RNAs is the so-called miRNA. Discovered in vertebrates less than a decade ago, these short (~22 nucleotides), single-stranded, endogenous RNA molecules potently inhibit the translation of specific mRNAs. This effect results from the binding of the so-called seed sequence near the 5′ end of the miRNA to its complementary target within the 3′ untranslated region of the miRNA molecule. Occasionally, perfect Watson-Crick pairing is achieved, and the miRNA is degraded (Figure). More commonly, imperfect pairing occurs, and translation is impaired without destruction of the genetic transcript. Each miRNA may regulate upward of 500 different genes. The genes of a cluster regulated by single miRNA commonly act together to modulate integrated pathways that subserv a biological response. Because of the promiscuity of the miRNA system, >30% of all human genes are predicted to be regulated by fewer than 1000 individual miRNAs. This intricate and highly conserved class of molecules plays a critical role in many pathological conditions, including vascular inflammation, arterial remodeling, smooth muscle plasticity, atherosclerosis, stem cell differentiation, and endothelial cell apoptosis.

miRNA Mechanism for Impaired Angiogenesis in Diabetes

In the current issue of *Circulation*, Caporali and colleagues have augmented our understanding of miRNA biology in the vascular pathophysiology observed in diabetes. They discovered that when endothelial cells were exposed to conditions that mimic hyperglycemia and tissue hypoxia, the cells expressed increased levels of miRNA-503. To determine whether there is a causal role for miRNA-503 in the impaired angiogenesis observed in diabetes, they forced the expression of this miRNA in endothelial cells. They observed a dramatic and deleterious effect of this miRNA on several processes central to angiogenesis, including endothelial proliferation, migration, and tube formation in the in vitro Matrigel model of angiogenesis. To determine whether this miRNA was operative in vivo, they studied its expression in animals and humans. They observed that miRNA-503 expression was increased after surgically inducing ischemia in the hindlimbs of diabetic mice. Notably, miRNA-503 was also elevated in the blood and the calf muscles of diabetic patients with advanced limb ischemia. To definitively show that this particular miRNA directly causes and is not just associated with impaired angiogenesis in diabetes, the authors injected into the ischemic hindlimb of diabetic mice an miRNA “ decoy” that contained multiple copies of the target miRNA binding site. By this technique, miRNA-503 was effectively scavenged and was no longer available to bind and inhibit its target mRNA. As predicted, they observed that antagonizing...
miRNA-503 resulted in a dramatic improvement in blood flow and postischemic angiogenesis, the first example of an miRNA-based intervention restoring physiological angiogenesis in diabetes.

The study left unanswered some questions regarding the mechanism of action by which miRNA-503 exerts its antiangiogenic effects. Caporali et al.8 report that the antiangiogenic miRNA-503 targets 2 well-known cell cycle–regulating genes, cyclin E and cdc25, findings that have been described previously in other cell types.21,22 These cyclin-related factors play an absolutely central role in the cell’s decision to undergo the G1-to-S transition, and govern critical cell fate processes such as differentiation, proliferation, and cellular senescence.23 Intriguingly, major genome-wide association studies for cardiovascular disease states have implicated polymorphisms in close proximity to 2 major cyclin-related factors,21,22 these cyclin-related factors provide a genetic mechanism of action by which miRNA-503 exerts its antiangiogenic effects.

Other miRNAs Participate in Angiogenesis

Before the work of Caporali and colleagues,8 other researchers found a role for miRNAs in regulating angiogenesis. Early reports revealed that Dicer (a critical miRNA-processing enzyme) led to a dramatic impairment in blood vessel network formation and embryonic lethality, which indicates a critical role of miRNAs in vasculogenesis.26 Subsequent studies have identified several potent proangiogenic and antiangiogenic miRNAs.27

For example, miR-126 is now known to target SPRED1 and PIK3R2, 2 critical inhibitors of the angiogenic cytokine VEGF.28 By reducing the expression of these antiangiogenic cytokines, miR-126 enhances capillary network stability and flow-induced vascular remodeling in zebrafish models of blood vessel development.29 Other vasculogenic miRNAs (and their target genes) have also been described, including Let-7 (thrombospondin-1),26 miR-210 (ephrin A3),30,31 and the miR-17-92 cluster (thrombospondin-1 and connective tissue growth factor),32 among others. Conversely, several antiangiogenic miRNAs have also been uncovered, including miR-221/222, miR-92a, and miR-509c, which inhibit tube formation, vessel growth, and other endothelial cell functions by reducing c-kit, integrin subunit-α5, and hypoxia-inducible factor-1α signaling, respectively.33–35

How these miRNAs interact to modulate angiogenesis in health and disease is not known. It is likely that some are more prominent than others in specific disease states. Furthermore, it is not clear which, if any, of these miRNAs underlie other molecular mechanisms featured in diabetic pathophysiology (eg, generation of reactive oxygen species and impairment of NO bioactivity). A striking feature of diabetes mellitus is the heterogeneity of angiogenic dysregulation. For example, VEGF is upregulated in the diabetic eye, whereas VEGF signaling is impaired in the peripheral vasculature.36 Could differential tissue regulation of miR-503 and/or other angiogenesis-modulating miRNA explain the paradox of attenuated angiogenesis in the diabetic leg ulcer, which coexists with the proliferative angiogenesis observed in the retina of the same diabetic patient? There remain many questions of scientific interest and clinical relevance to be addressed on this emerging research front. Chief among these is the question of how we will modulate miRNA expression for therapeutic purposes. To be sure, further developments in this field are likely to uncover novel methods of promoting (eg, vascular regeneration in ischemia) or inhibiting (eg, tumor growth in metastasis) angiogenesis via manipulation of this epigenetic system.

Disclosures

Dr Cooke is an inventor on Stanford University patents related to therapeutic modulation of angiogenesis by agonists or inhibitors of the nicotinic acetylcholine receptors.

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