Neovascularization Driven by MicroRNA Delivery to the Endothelium

Henry S. Cheng, Jason E. Fish

Atherosclerotic disease can lead to severely debilitating conditions, such as myocardial infarction and peripheral artery disease, which are associated with poorly perfused tissue. Despite the initial promise of proangiogenic gene therapy or cell-based approaches to rectify ischemic diseases in preclinical models,1–3 large randomized placebo-controlled clinical trials have revealed only modest effects, at best.4,5

Alternatively, rather than delivering proangiogenic genes (such as vascular endothelial growth factor [VEGF]) or proangiogenic cells, manipulating pathways that lie downstream of receptors for angiogenic factors may provide a more robust outcome. In this regard, microRNAs (miRs) are appealing as therapeutic targets/agents for several reasons: (1) miRs often repress multiple targets within common or complementary pathways, allowing for a strong synergistic effect that is less likely to induce resistance compared with a single therapeutic target, (2) miRs are short ≤22 nucleotide sequences that can be easily inhibited or overexpressed, and (3) miR sequences are highly conserved across multiple species, aiding the transition between preclinical animal models and clinical trials in humans.6

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Extensive research has revealed that miR-126-3p, an endothelium-enriched miR, promotes angiogenesis and vascular stability by targeting distinct repressors (ie, sprouty-related, EVH1 domain containing 1 [SPRED1] and phosphatidylinositol 3-kinase regulatory subunit 2 [PIK3R2]) of the VEGF pathway.7–10 Interestingly, miR-126 delivery to endothelial cells (ECs) via circulating microparticles or apoptotic bodies promotes vascular repair in animal models,11,12 providing a strong impetus to develop miR-126–directed proangiogenic therapies. One of the major challenges in using miRs as a therapeutic is the difficulty in limiting delivery to the appropriate cell type or tissue. Although therapeutic delivery of miRs to ECs has recently been accomplished using intravenous injection of liposome-encapsulated miRs in animal models,13 this approach does not allow for delivery to specific regions of the vasculature, and delivery is by no means specific for ECs, as circulating leukocytes can take up the miR, and large quantities are also delivered to the liver, kidney, and spleen. Ultrasound-targeted microbubble destruction (UTMD) may bypass the challenges of cell specificity by using ultrasound to rupture miR-loaded microbubbles at the site of interest to promote local delivery. UTMD is a noninvasive method that has been used for the delivery of plasmids or miRs preferentially to ECs in animal models of ischemic disease.2,14,15

Endo-Takahashi et al14 recently used UTMD to deliver miR-126 to the vasculature of mice with hindlimb ischemia and found a beneficial effect, including an increase in the expression of several angiogenic factors, coupled with improvement in blood flow. However, their study neither assessed the mechanism(s) of the proangiogenic effects of miR-126 nor did they assess the maturity and stability of the formed vessels.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Cao et al16 have provided further mechanistic insight into therapeutic modulation of angiogenesis via UTMD-mediated delivery of miR-126. They demonstrate specific delivery of miR-126 to the ECs and pericytes of vessels in ischemic rat hindlimb muscle exposed to ultrasound and downregulation of known miR-126 targets, such as SPRED1 and PIK3R2. Importantly, levels of miR-126 and target genes are not altered in the liver and spleen, common off-target organs. MiR-126 delivery enhances vessel length, vascular density, and perfusion ≤14 days post UTMD (the end point of the study). Of particular importance, miR-126 delivery also enhances the calibre of the vessels that are formed, as well as their pericyte coverage: evidence of neovessel maturation. At the molecular level, they have found that miR-126 overexpression enhances TIE2 phosphorylation in response to ANG1 treatment and that this effect is dependent on the regulation of PIK3R2 by miR-126 (Figure), building on the observations of others.17 This is a key finding because proangiogenic therapy using VEGF overexpression produces primarily immature vessels that can regress. However, subsequent overexpression of ANG1 can enhance the maturation and stability of neovessels formed in response to VEGF.18

Thus, it seems that miR-126 is able to drive coordinated vascular formation and maturation through augmented signaling through the VEGFR2 and TIE2 receptors (Figure), achieving similar effects observed with temporal delivery of VEGF and ANG1 expression vectors.18

To apply UTMD miR delivery as a therapy, it is critical to understand the duration of miR delivery and action. Cao et al16 have observed that miR-126 transfection occurs from 3 hours to 3 days after ultrasound, with target gene knockdown lasting 3 days. Although target genes return to normal levels by 14
days post UTMD, a sustained effect on vascular formation is observed. Because UTMD is a minimally invasive treatment, repeated treatments would be expected to enhance and prolong miR delivery, target gene knockdown, and neovascularization.

Indeed, repeated rounds of UTMD (days 14, 16, and 18 post injury) provide a greater effect on the level and duration of target gene suppression, with decreased PIK3R2 protein levels persisting even 14 days after the first injection (ie, 28 days post hindlimb ischemia). Vascular perfusion is also enhanced. Thus, it seems to be feasible to repeatedly deliver miR-126 to the same tissue to prolong the neovascularization response.

From a therapeutic point of view, UTMD is appealing and provides clear advantages over other delivery methods, such as the use of liposomes or viral expression vectors because it allows temporal and spatial control of miR delivery. Further work to define the spatial resolution of this approach is warranted. This will allow for the determination of whether ischemic regions of the infarcted heart might be targeted by angiogenic miRs, while avoiding delivery to healthy regions of the heart. Although the results of Cao et al16 have provided optimism that UTMD approaches might be beneficial for human ischemic disease, there are several limitations of this study that should be noted. Many experimental therapies that have shown promise in preclinical models do not have similar efficacy in human clinical trials.19 This may be due in part to the nature of the preclinical models themselves. Although experimental animals are typically young and healthy, patients with ischemic diseases, such as myocardial infarction and peripheral vascular disease, are not typically young and have significant comorbidities, such as hypercholesterolemia, obesity, and diabetes mellitus. It is not clear how these comorbidities might influence the effectiveness of miR-126 delivery in humans. The hindlimb ischemia model used by Cao et al16 is considered a chronic ischemia model because 2 weeks after ligation before therapy is initiated. However, in many human patients, ischemia may be present for many months or years. This may create a vastly different substrate with which miR-126 needs to act on to elicit growth of a new functional vasculature. Furthermore, this delivery modality requires a minimum level of blood flow to deliver the microbubbles to the desired tissue. Some areas of tissue ischemia in humans may be nearly completely obstructed, preventing the use of this treatment strategy. Finally, the safety of repeated UTMD over time will also need to be assessed. MiR-based therapeutics have garnered substantial interest, and the first clinical trials showing positive effects are emerging.20 The findings by Cao et al16 reveal additional tools that will provide further opportunities to bring miR-based therapies to the clinic by providing an effective way to deliver angiogenic miRs to specific regions of the vasculature.

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References


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