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MiRrored Regulation of KLF2 and KLF4

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A healthy organism demands much from its endothelium; constance in homeostatic control of blood fluidity, vascular tone, interaction with circulating blood cells, maintenance of barrier functions, and, when appropriate, creation of new blood vessels. Breakdown in any of these functions, anywhere in the 60,000 miles of vessels in the human body, leads to or exacerbates a vast array of diseases including those plaguing modern man: atherosclerosis, diabetes, hypertension, cancer, and clotting disorders. In recent years two endothelial transcription factors, Kruppel-like factor 2 (KLF2) and Kruppel-like factor 4 (KLF4), have garnered attention as guardians of endothelial health. Both KLFs confer vascular protection via regulation of gene programs resulting in an anti-inflammatory, anti-coagulant, anti-adhesive, anti-oxidant state of the endothelium.[1-4] Thus, increased knowledge of the regulation of these regulators is of great interest. In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* Fang and Davies demonstrate that a single microRNA, miR92, regulates both KLF2 and KLF4 — a finding that has exciting scientific and potentially therapeutic implications.

Atherosclerosis develops first in curved or branched portions of vessels where nonlaminar, oscillatory blood flow occurs. Such atheroprone regions include the bifurcation of the common carotid into the internal and external branches and the internal curvature of the aortic arch.[5] Atheroresistant areas of the vasculature are those exposed to laminar flow, like the external curvature of the aortic arch and the descending aorta. *In vitro* studies have demonstrated that KLF2 and KLF4 are potently upregulated by laminar flow.[1, 2, 6] It has been of considerable interest that these KLFs are highly expressed *in vivo* in atheroresistant regions while present in significantly lesser amounts in areas prone to atherosclerosis,[6] and in fact Atkins et al. have shown that deficiency of KLF2 augments experimental atherosclerosis.[7] Laminar flow-mediated mechanisms that increase KLF2 or KLF4 expression have been identified and include activation of the AMPK-dependent MEK5/ERK5/MEF2 pathway,[8, 9] deinhibition of histone deacetylase 5 (HDAC5)-mediated transcriptional silencing,[10] and stabilization of KLF2 mRNA.[11] Wu et al. recently reported that miR92a levels are decreased in the endothelium of atheroprone regions leading to increased KLF2 levels via enhanced mRNA stability,[12] and herein Fang & Davies suggest the same for KLF4.

Fang & Davies use TNFα treatment of endothelial cultures as their *in vitro* model for the inflammation seen at atheroprone regions of the vasculature and show that deficiency of miR92a in this context allows for enhanced KLF4-mediated modulation of inflammatory targets. This is a reasonable model for atherosclerosis as NF-κB activity (the pathway activated by TNFα) is enhanced in atheroprone regions.[13] Commonality of regulation and function for KLF2 and KLF4 has often been described. In addition to the post-transcriptional regulation by miR92a, upstream regulators include laminar flow, HMG-CoA inhibitors (statins), proteasome inhibitors, and agents with anti-oxidant properties.[1, 6, 8, 14-19] However, KLF2 and KLF4 respond quite distinctly to exposure to pro-inflammatory agents such as TNFa; KLF2 levels are decreased whereas KLF4 is upregulated. For this reason, the limited effect by KLF2 on modulation of inflammatory targets as seen by Fang and Davies in this paper (as opposed to the significant effects by KLF4) may not be recapitulated fully under flow conditions, where additional stimuli are present. It will be of significant interest to see what effects on atherosclerosis development are brought about by *in vivo* endothelial KLF4 deficiency.

The results of this study may also have therapeutic implications. Traditionally, transcription factors are viewed as “undruggable targets.” We find this argument weak, particularly as drugs targeting PPARγ function are potent therapeutics in the treatment of diabetes. The findings of Fang & Davies coupled with those of Wu et. al. raise the possibility that inhibition of miR92a could be exploited to augment endothelial KLF levels and thereby confer favorable homeostatic effects to the vasculature. miR92a is one of six mature products of the polycistronic miRNA cluster miR-19-92, also called oncomir-1. Expression of the polycistron has been implicated in the pathogenesis of hematopoetic and solid tumors as well as in normal development. Endothelial effects of oncomir-1 include promotion of tumor angiogenesis.[20] However individual miRNAs of the oncomir-1 cluster appear to have differential effects on angiogenesis,[21] and forced overexpression of miR92a alone has been shown to inhibit angiogenesis in mouse models of limb ischemia.[22] Thus alteration of miR92a levels and not regulation of the oncomir-1 cistron would need to be the focus of therapy. Even so, a single microRNA can regulate hundreds of targets. The miRanda software predicts 300 targets for miR92a;[23] however, such algorithms appear to predict only ∼50% of targets detected by microarrays and other experimental techniques.[24] This suggests that targeting miR92a even with a specific antagomir or siRNA may produce unexpected effects. Nonetheless, therapeutic miR inhibition is feasible, and for miR122 already in Phase I clinical trials.[25] Certainly, given the potentially enormous impact on vascular health of augmenting endothelial KLF2 and KLF4, efforts to manipulate miR92a are clearly worthy of pursuit.

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