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The battle of the bulge: miR-195 vs. miR-29b in aortic aneurysm

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Abdominal aortic aneurysm (AAA) is a potentially lethal condition, capable of progressing to acute rupture – a catastrophic event with an 80% mortality risk. A conservative estimate places annual deaths due to AAA in the U.S. at ~15,000, although the actual burden is likely higher¹. These dilations are typically found within the infra-renal aortic segment, but are only rarely symptomatic, causing them to remain undiscovered until they are either identified through coincident imaging, or rupture occurs. Among the risk factors for AAA are male sex, advanced age, genetic predilection, and a history of tobacco use. No pharmacological approach in humans to-date has successfully decreased AAA expansion or prevented rupture². While surgery and endovascular stent-grafting are highly effective in preventing death from larger AAAs, they are complex procedures with multiple potential complications. How then, might the battle against AAA be better waged?

MicroRNAs are intricately woven into a web of epigenetic pathophysiologic regulation. Modulation of any given microRNA can alter the expression of dozens of target genes, including entire functional gene networks, thereby affecting the progression of a wide array of disease phenotypes. In recent reviews examining the role of microRNAs in AAA, we noted their remarkable potential, both to improve risk-stratification and diagnosis, and to alter vascular disease therapeutically. In this vein, exciting findings have been published for several microRNAs including: miR-21, miR-26a, the miR-17-92-cluster, miRs-221/222, miR-133, miR-126, miR-143/145, miR-146a, miR-155, and miR-29b^{3, 4}.

Of these candidates, the last (miR-29b) appears particularly promising. Remodeling of the extracellular matrix (ECM) within the aortic adventitia and media is crucial for AAA progression, characterized by elastin fragmentation and loss and increased collagen turnover. MiR-29b targets include numerous collagen genes and elastin. Further, miR-29b modulation *in vitro* and *in vivo* can alter matrix metalloprotease (MMP) activity. MiR-29b is differentially regulated in animal models of aneurysm and in human AAA tissue, and inhibition of miR-29b in murine models of AAA and Marfan syndrome has led to diminished aneurysm progression (while overexpression increases aneurysm growth and rupture rate)^{5–7}.

In this issue of *Circulation Research*, Zampetaki et al. examine miR-195, a member of the miR-15 family known to share many of the same targets as miR-29b^{8, 9}. They found that miR-195 (alone of the miR-15 family) was increased in aneurysmal aortic tissue from Angiotensin II(AngII)-treated *apoE*^{–/–} mice. AngII has previously been shown to induce or inhibit miR-15a, -15b, -16-1 and -16-2 in either rat or human smooth muscle cells (SMC),

but not miR-195^{10, 11}. Further, while significant downregulation of miR-15a, miR-195 and miR-497 has been observed in tissue from dissected human thoracic aorta when compared with normal aorta, Pahl et al.'s expression profiling of human AAA tissue did not identify any differentially regulated miR-15 family members^{12, 13}.

In human aortic SMC, miR-195 mimic was able to suppress elastin expression, but unlike miR-29b, caused a non-significant increase in MMP9 activity and an increase in MMP2 expression. The authors then performed proteomic studies of SMC, confirming that miR-195 regulates numerous ECM elements, although not to the same extent as miR-29b (especially in terms of collagen repression).

More disappointing were attempts at *in vivo* inhibition of miR-195 in the mouse AAA model. While clear suppression of miR-195 in the aorta was achieved, correlating with increases in expression of elastin and collagens, there was no significant impact on aneurysm progression or subject survival. (No *in vivo* miR-195-mimic experiments were performed.) Immunohistochemistry showed increased *Mmp9* expression in anti-miR-195 transfected aortae. In contrast, and confirming published data using the same model, anti-miR-29b led to improved survival and slowed aortic growth. Maegdefessel et al. also demonstrated that systemic anti-miR-29b lowered MMP2 and MMP9 activity and expression *in vivo*⁷.

Zampetaki et al. noted that antagomiR-29b had minimal effects on miR-29b levels in an aortic isograft model. This supports the conclusion in Maegdefessel et al. that LNA-anti-miR-29b is not readily taken up by uninjured vessel wall. However, in both studies, uptake within the affected suprarenal aorta (AngII murine model) was sufficient to alter disease progression and target expression. In contrast anti-miR-195 diffused more readily into uninjured vasculature, but did not abrogate disease. This is unusual, as in our experience intact endothelium often resists LNA-miR uptake. The authors attribute the contrasting *in vivo* results between anti-miR-195 and anti-miR-29b to their inverse regulatory effects on MMP activity. Given the known association between MMP activity and AAA severity and progression, this may well be the case¹⁴.

Interestingly, in addition to its role in ECM regulation, miR-195 is a known tumor suppressor, which has been shown to demonstrably inhibit growth and proliferation, promote apoptosis, and inhibit cellular migration in various tissues and cell types¹⁵. In contrast another microRNA – miR-21 – inhibits tumor suppressors, eliciting the opposite cellular responses from those attributed to miR-195, and pre-miR-21 administration has significantly curtailed murine AAA growth¹⁶. It might have been expected that anti-miR-195 would therefore have similar effects. However, miR-195 is also known to suppress angiogenesis¹⁷. Angiogenesis inhibition is believed to limit AAA progression, which might therefore have further undermined the effectiveness of anti-miR-195¹⁸.

Zampetaki et al. also suggest an intriguing role for miR-195 as an AAA biomarker, which miR-29b is unlikely to match (as it was barely detectable in human plasma samples). Circulating miRNAs are stable in human blood, and detectable and measurable with high sensitivity and specificity, suggesting they might make effective AAA biomarkers^{19, 20}.

Clinical studies have demonstrated changes in miRNA levels in association with cardiovascular disease phenotypes, although these have often been underpowered or lacking in matched controls^{21, 22}. The authors examined 16 miRNAs in plasma from 73 participants from an aneurysm screening program, finding that miR-195 was inversely correlated with aneurysm size and disease classification. While 4 other miRs also showed such association, they were closely correlated with miR-195, and after proper adjustment their association with aortic size diminished. Intriguingly, miR-133a and miR-145 emerged as significant after adjustment for miR-195, suggesting that further studies involving a combination approach might yield a robust biomarker panel. As in the work cited above, the sample size queried was quite small for a biomarker study, and these findings will require extensive verification and replication in larger data sets to prove clinical utility.

Despite the similar target profiles of miR-29b and miR-195, it remains to be seen whether the first may someday triumph on the field of therapeutic AAA abrogation, and whether the latter will fulfill its promise as a predictive biomarker of AAA progression.

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