

Micromanaging restenosis by therapeutic inhibition of miR-92a

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This editorial refers to ‘Inhibition of miR-92a improves re-endothelialization and prevents neointima formation following vascular injury’ by J.-M. Daniel *et al.*, pp. 564–572, this issue.

The adverse consequences of procedure-associated endovascular injury may limit the long-term benefits of coronary interventions like angioplasty and stent insertion. Damage to the artery's endothelium and impaired re-endothelialization, also caused by the use of non-selective anti-proliferative drugs, may promote the development of restenosis—a pathological condition characterized by smooth muscle cell (SMC) hyperplasia, vessel thickening and lumen narrowing, and impaired blood flow—in a significant proportion of the patients. Reducing the occurrence of restenosis after coronary interventions thus remains an important medical need.¹ In this issue of *Cardiovascular Research*, Daniel *et al.*² report that both genetic and molecular (therapeutic) approaches to inhibit microRNA (miRNA)-92a facilitate arterial re-endothelialization and prevent restenosis in a mouse model of femoral artery injury.

miRNAs are a class of small non-coding RNAs that fine-tune gene expression at the post-transcriptional level. Several miRNAs, including miR-126, miR-132, miR-222, and miR-92a, have been implicated in the regulation of endothelial cell (EC) biology.³ miR-92a is a member of the miR-17-92 cluster, which encodes six distinct miRNAs broadly involved in physiological and pathological processes such as cell proliferation, development, immunity, and tumorigenesis.⁴ While being deregulated in several leukaemias and solid tumours, miR-92a also functions as a negative regulator of EC proliferation, angiogenesis, and vascular repair. Therapeutic modulation of miR-92a activity in ECs may, therefore, rescue the damaged endothelium after coronary interventions.^{5–8}

Daniel *et al.*² employed a mouse model of wire-induced injury of the femoral artery to analyse the temporal and cellular expression of miR-92a after vascular damage. They found that miR-92a levels increased post-injury and peaked at Day 10, a time-point when SMC hyperplasia was already evident in the injured artery. The analysis of cultured ECs and SMCs, as well as intact or endothelium-denudated arteries, suggested that ECs and not SMCs were the main source of miR-92a in the injured arteries. Moreover, transfection of miR-92a inhibited

vascular endothelial growth factor-A (VEGFA)-induced EC proliferation and migration, but did not affect platelet-derived growth factor-BB (PDGFB)-induced SMC proliferation or migration, indicating that the functions of miR-92a are largely EC-autonomous. The authors then used two loss-of-function strategies to attenuate miR-92a activity in the damaged arteries. Both the systemic delivery of locked nucleic acid (LNA)-modified anti-miR-92a oligonucleotides⁹ and the conditional knockout of miR-92a in TIE2⁺ ECs stimulated re-endothelialization and decreased SMC hyperplasia and inflammatory—macrophage infiltration in the femoral artery after wire-induced injury (Figure 1). These data suggest that suppression of endothelial miR-92a activity promotes arterial re-endothelialization and limits SMC hyperplasia, at least in part, through direct pro-proliferative effects on ECs.² Consistent with these findings, previous studies showed that inhibition of miR-92a enhances VEGFA-induced EC proliferation by activating mitogenic ERK and JNK signalling.⁶

Besides direct pro-proliferative effects on ECs, inhibition of miR-92a may attenuate experimental restenosis through additional mechanisms. Among the validated targets of miR-92a are the deacetylase sirtuin-1 (*Sirt1*) and integrin- α 5 (*Itga5*). SIRT1 is highly expressed in the angiogenic vasculature and promotes sprouting angiogenesis, whereas ITGA5 enables migration, pro-angiogenic signalling, and angiogenesis of ECs by modulating their interactions with the extra-cellular matrix.¹⁰ Daniel *et al.*² observed increased expression of both SIRT1 and ITGA5 in the arterial ECs of anti-miR-92a-treated mice 2 weeks post-injury, suggesting that therapeutic inhibition of miR-92a stimulates re-endothelialization, at least in part, by de-repressing both pro-angiogenic factors. miR-92a also targets the transcription factors Krüppel-like factor-2 (KLF2) and 4,¹¹ which confer anti-inflammatory and atheroprotective properties to the endothelium. De-repressed KLF2 and 4 may operate to down-regulate the expression of leucocyte adhesion molecules on ECs, hence limiting inflammatory cell infiltration, and to enhance endothelial nitric oxide (NO) synthetase (NOS3/eNOS) activity, which inhibits SMC proliferation through NO production.^{6,11,12} Thus, therapeutic inhibition of miR-92a may initiate an anti-atherosclerotic programme in ECs that limits inflammatory cell infiltration and SMC proliferation in the healing arteries.^{5–8}

Daniel *et al.*² and previous studies^{5–8} employed anti-miR-92a oligonucleotides delivered systemically in animal models of vascular injury.

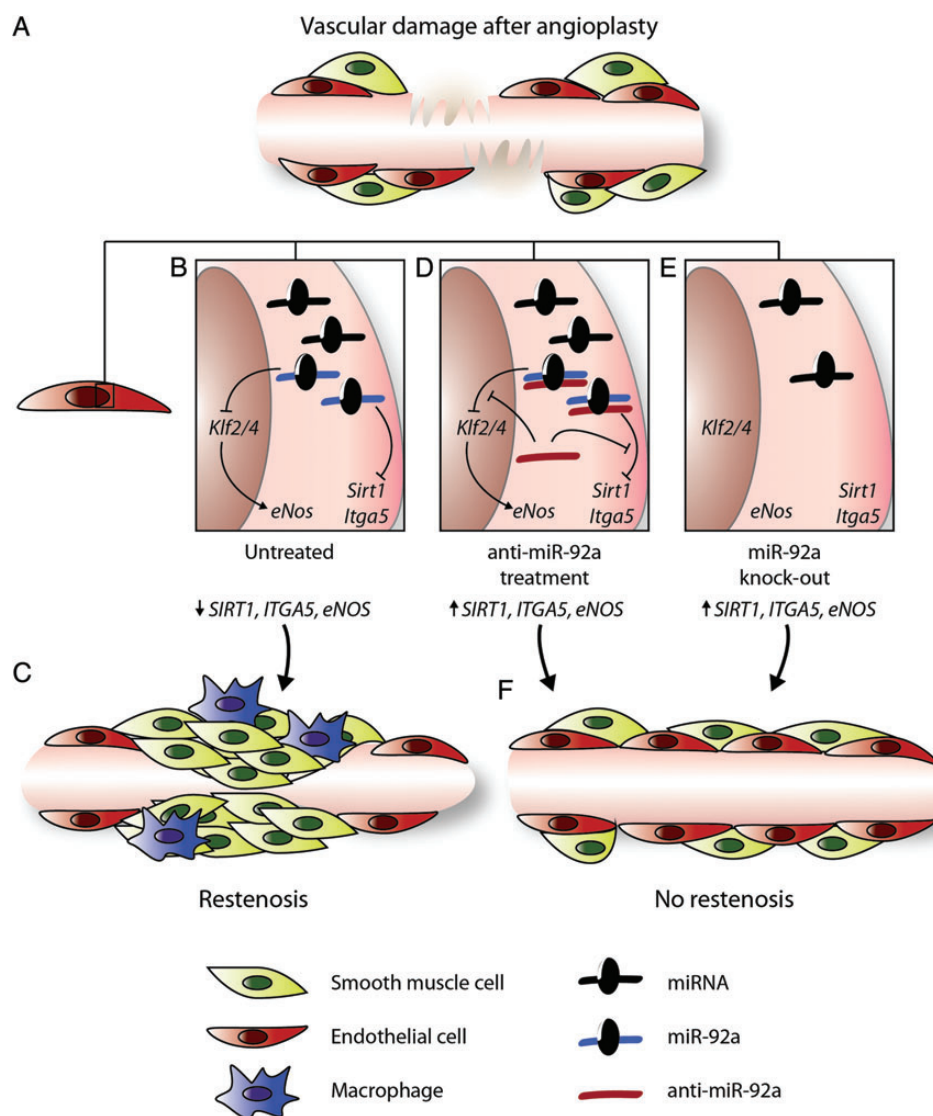


Figure 1 Inhibition of restenosis by therapeutic or genetic miR-92a targeting. Endothelial damage consequent to coronary interventions (A) up-regulates the expression of miR-92a, which directly targets the pro-angiogenic and anti-inflammatory factors *Sirt1*, *Itga5*, and *Klf2/4* (B), inhibits EC proliferation, and promotes the development of restenosis (C). Systemic treatment with anti-miR-92a oligonucleotides (D), or the genetic deletion of miR-92a in ECs (E), restores *Sirt1*, *Itga5*, and *Klf2/4* levels in the healing endothelium, stimulates re-endothelialization, decreases SMC hyperplasia (via increased eNOS activity and NO production), and ultimately limits the development of restenosis (F). Please see text for references.

Because systemic anti-miR oligonucleotides target multiple organs and cell types,^{9,13} this approach may have altered miR-92a activity also in non-ECs. Furthermore, anti-miR-92a oligonucleotides may potentially target several mature miRNAs. Indeed, miR-92a, miR-92b, and miR-25 belong to the same miRNA family and thus share the seed-sequence used to design anti-miR oligonucleotides. Moreover, two mature miR-92a sequences exist that are expressed from two distinct genetic loci, miR-17-92 (encoding miR-92a-1) and miR-106-363 (encoding miR-92a-2). Based on the above, non-EC autonomous effects and potential co-targeting of distinct miRNA species could not be formally excluded in previous studies.^{5–8} To address these issues, Daniel *et al.*² employed conditional knockout strategies either targeting the miR-92a-1 gene broadly in haematopoietic cells or specifically in TIE2-lineage cells, which comprise both ECs and haematopoietic cells.¹⁴ By this comparative analysis, the authors unequivocally showed that the

miR-92a sequence encoded by the miR-17-92 locus is functionally important in the endothelial, but not in the haematopoietic, lineage.

Although not yet broadly tested in the clinic, anti-miR therapeutics are being successfully used in animal models to experimentally suppress miR activity.^{9,13} For example, systemic anti-miR-33 therapy holds promise for the treatment of dyslipidaemias and associated vascular/cardiac diseases, as shown by preclinical studies in animal models.¹³ Remarkably, a phase II clinical study recently demonstrated the efficacy of a LNA-modified anti-miR-122 for the inhibition of hepatitis C virus (HCV) replication in patients.¹⁵ It should be noted that both the pharmacokinetic properties and the intravenous route of administration of anti-miRs facilitate targeting of the liver, which may explain the reported success of anti-miR-122/33–based treatments in animal models and patients.¹³ On the other hand, it is currently unclear whether systemic administration of anti-miR-92a therapeutics would efficiently target the arterial

endothelium of patients undergoing coronary interventions. Also, potential toxicities associated with the systemic down-regulation of miR-92a, particularly in non-ECs, should be considered. Although miR-92a knockout mice are viable and fertile, they show increased embryonic lethality as well as growth and skeletal defects.¹⁶ To alleviate these concerns, drug-eluting stents¹ that deliver anti-miR-92a locally to the healing endothelium should be tested in large animal models to explore the advantages and disadvantages of this approach compared with systemic administration.

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