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# Secreted miRNAs suppress atherogenesis

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## Abstract

Endothelial–vascular smooth muscle cell communication has a critical role in cardiovascular homeostasis and the pathogenesis of atherosclerosis. A study now demonstrates extracellular-vesicle-mediated transfer of the atheroprotective microRNAs miR-143/145 between endothelial and vascular smooth muscle cells, providing compelling evidence that intercellular transport of miRNAs can influence a pathological process, namely atherosclerosis.

Signalling and communication between endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) is critical for angiogenesis during embryonic development, as well as homeostasis and adaptation of the adult vasculature. Alterations in EC–VSMC communication have been implicated in the pathogenesis of common vascular pathologies, including atherosclerosis, arterial aneurysm and dissection. EC–VSMC signalling is also involved in tumour angiogenesis and metastasis[1]. However, a fundamental question remains as to how the myriad of signals transduced by the ECs efficiently and differentially communicate to VSMCs, both during development and in response to haemodynamic stimuli in the adult.

Secreted growth factors are important regulators of the EC–VSMC interface. During embryonic angiogenesis, EC-mediated recruitment of pericytes to capillaries and VSMCs to arteries and veins is required for vessel maturation and stabilization. Platelet-derived growth factor B (PDGF-B) is secreted from the endothelium of angiogenic sprouts, where it serves as an attractant for co-migrating mural cells (a precursor to pericyctes and VSMCs) expressing the PDGFRβ receptor[2]. PDGF-B stimulates proliferation of VSMCs and induces mural cell fate in undifferentiated mesenchymal cells[2]. Angiopoietin-Tie receptor signalling occurs in the reverse direction, as the secretion of angiopoietin-1 by mural cells activates the Tie2 receptor on ECs, promoting pericyte adhesion and tightening of endothelial cell junctions[3]. Transforming growth factor-β ligands and their receptors stimulate mural cell induction, differentiation, proliferation and migration to the vessel wall[4]. Conditional ablation of the *Tgfbr2* gene (transforming growth factor-β receptor 2) in either ECs or VSMCs results in near-identical vascular phenotypes, highlighting the two-way flow of


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communication between ECs and VSMCs in the developing vasculature[5]. However, EC–VSMC communication is not restricted to paracrine mechanisms; for example, Notch and Wnt signals are transduced directly through cell-cell interactions mediated by EC ligands and VSMC receptors, and vice versa. On page 249 of this issue, Hergenreider *et al.*[6] identify yet another means of EC–VSMC communication — secreted vesicles carrying miRNAs.

Blood vessels are constantly subjected to haemodynamic forces, including hydrostatic pressure, cyclic stretch and fluid shear stress, induced by pulsatile blood pressure and flow[7]. Shear stress, which is the component of frictional forces arising from blood flow acting parallel to the vessel luminal surface, is borne predominantly by ECs. Fluid shear stress modulates the structure and function of ECs by activating, or repressing, sets of genes, including many that have been implicated in the pathogenesis of atherosclerosis as well as other heritable and acquired vascular diseases. The exposure of ECs to shear stress or laminar flow induces the expression of a set of atheroprotective genes such as *eNOS* (endothelial nitric oxide synthase). Conversely, decreased shear stress at sites of disturbed laminar flow causes the downregulation of atheroprotective genes and the concomitant upregulation of a set of pro-atherogenic genes. One unanswered question concerns how physical forces influencing EC phenotype are transmitted to VSMCs, thus modulating their phenotype in segments of the vasculature predisposed to atherosclerosis and other vascular diseases.

Kruppel-like factor 2 (KLF2) is a lineage-restricted member of the Kruppel family of transcription factors that serves as a key ‘molecular switch’, regulating important aspects of vascular function and disease[8]. KLF2 expression is rapidly induced in ECs exposed to fluid shear stress. Reduced KLF2 is observed in humans at sites of disturbed laminar flow, such as the bifurcation of the aorta, and branch points of the carotid, iliac and coronary arteries that are predisposed to atherosclerosis. KLF2 lies upstream in the transcriptional program, promoting the atheroprotective phenotype in ECs (ref. 7). It induces expression of anti-inflammatory and anticoagulant proteins, and inhibits pro-inflammatory molecules including NF-κβ and its target genes. The combined effects of KLF2 and Nrf2, a KLF2-regulated transcription factor responsible for anti-oxidant gene expression, are thought to account for approximately 70% of shear-stress-induced changes in EC gene expression[9]. *Klf2*$^{-/-}$ null mutant embryos survive only until embryonic days 12.5-14.5, succumbing to intra-embryonic and intra-amniotic haemorrhage attributable to a block in VSMC recruitment to the vessel wall, leading to aneurysm and arterial rupture[10]. EC-restricted knockout of the *Klf2* gene leads to high-output heart failure caused by a marked decrease in peripheral resistance[11]. However, the mechanism by which KLF2 regulates arterial tone and vascular stability during embryonic development has been perplexing.

Hergenreider *et al.* now elucidate a previously unidentified pathway linking endothelial KLF2 expression to regulation of VSMC phenotype[6]. In 2009, three groups independently reported that miRNAs 143 and 145 influenced VSMC phenotype[12-14]. However, the source of miR-143 and miR-145, and regulation of the miR-143/145 complex, remained largely unexplored. Recent studies have revealed that both mRNAs and miRNAs can be secreted by cells and transported intercellularly by exosomes and other types of extracellular vesicles. For example, exosomes derived from glioblastoma cells were found to transport miRNAs to

target cells and influence their phenotype[15], and microvesicles from bone marrow and mesenchymal cells were found to transport bioactive miRNAs to target cells[16]. Recently, high-density lipoproteins from human plasma were found to contain a range of miRNAs with biological activity on target cells[17]. Thus, a paradigm is evolving in which cells can secrete certain miRNAs in extracellular vesicles that can be transported in interstitial fluid to nearby cells or can hijack plasma lipoproteins for plasmabased transport, with uptake by target cells and subsequent effects on their phenotype.

As illustrated in Fig. 1, the report by Hergenreider *et al.* provides a compelling demonstration of intercellular communication between ECs and VSMCs mediated by extracellular vesicles bearing miRNAs[6]. Having previously demonstrated that miR-143 and miR-145 regulate VSMC phenotype[12], the authors noted that shear stress upregulated expression of the two miRNAs in endothelial cells through a KLF2-dependent mechanism both *in vitro* and *in vivo*. KLF2-binding sites were identified in the promoter of miR-143/145, and the authors observed direct promoter activation by either KLF2 or shear stress in reporter assays. Hergenreider *et al.* went on to show that KLF2-expressing ECs secrete extracellular vesicles comparable in size to exosomes (as determined by electron microscopy) that are enriched in miR-143 and miR-145. Using an EC-VSMC co-culture system, they demonstrated transfer of these miRNAs from ECs to VSMCs and, strikingly, a clear effect of these miR-NAs in reducing the mRNA abundance of known target genes in the VSMCs. VSMC gene expression and phenotype were altered in a manner predicted to be atheroprotective. This included suppression of KLF4 and Elk1, which suppress VSMC differentiation in response to oxidized phospholipids[18]. The authors proved this was not simply an *in vitro* phenomenon by isolating vesicles from the media of cultured KLF2-expressing ECs and repeatedly injecting them into atherosclerosis-prone mice, demonstrating a reduction in the disease. This result was dependent on expression of both KLF2 and miR-143/145 in the cultured ECs that served as the source of extracellular vesicles.

This exciting finding has important implications for vascular biology and the broader miRNA scientific community. For vascular biologists, it provides a plausible mechanistic link between laminar flow-induced endothelial KLF2 expression and atheroprotective VSMC phenotype, the nature of which has been uncertain. This means of EC-SMC communication and signaling also helps to provide molecular details that may in part underlie the well-established, but as yet poorly understood, association between endothelial shear stress and atherosclerosis. For the exploding field of miRNA biology, this report is one of the best demonstrations to date of intercellular transport of specific miR-NAs via extracellular vesicles, with effects on target cells that go beyond the demonstration of changes in mRNA abundance to show unequivocal impact on cell phenotype and pathogenesis of disease. In light of these results, miRNAs must be considered, alongside proteins, lipids and metabolites, as intercellular biological mediators, providing a potential explanation for the currently unexplained ability of one cell type to communicate with and influence the phenotype of other cells.

Despite the elegant exposition of the pathway, however, many questions remain. How is the release of the vesicles by ECs regulated, and do they contain other mediators, miRNAs or

other, that modulate the effects of miR-143/145 or directly influence VSMC phenotype? How are the miRNAs taken up by VSMCs so that they avoid intracellular degradation and remain biologically active? If VSMC expression of endogenous miR-143/145 is normally high except in the setting of injury, how does EC-derived miR-143/145 influence VSMC phenotype? How might these results be exploited in the development of a novel therapeutic approach to atherosclerosis? What other processes during embryonic development and postnatal adaptation of the vasculature are regulated through vesicle-mediated transport and targeting of miRNAs? Now that this pathway of intercellular communication has been firmly established, it will be fascinating to see where it leads.

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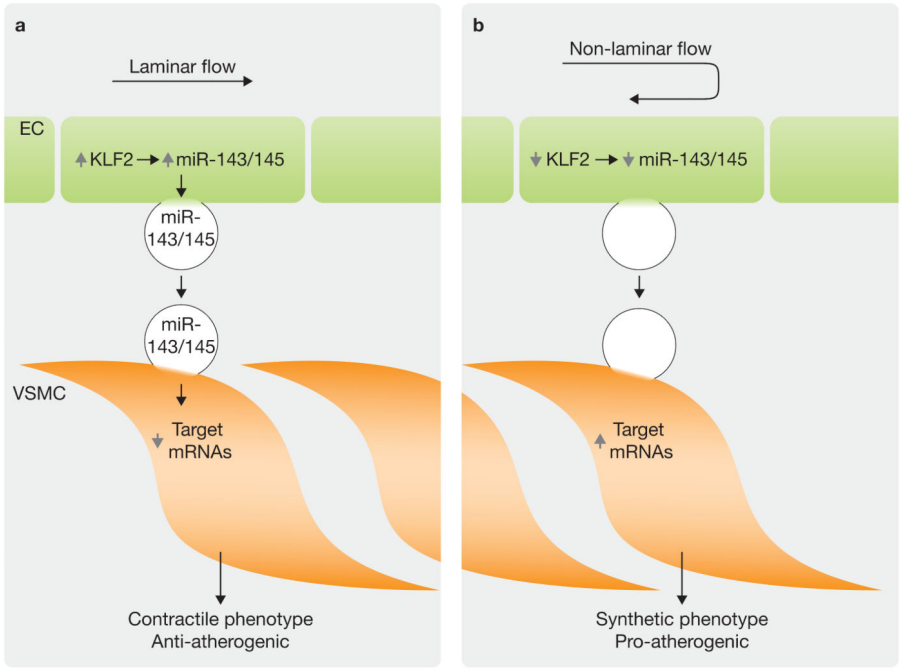


**Figure 1.**

Endothelial–VSMC communication through extracellular vesicles of atheroprotective miRNAs. (**a**) Under laminar flow conditions, endothelial KLF2 is upregulated. This leads to increased expression of miR-143/145 and transport of these miRNAs in extracellular vesicles to arterial VSMCs, where they repress specific target mRNAs involved in differentiation to the synthetic phenotype. As a result, the VSMCs are maintained in the contractile phenotype associated with normal arterial physiology and atheroprotection. (**b**) Under non-laminar or turbulent flow conditions, endothelial KLF2 is downregulated, leading to decreased expression of miR-143/145, decreased extracellular vesicle transport to VSMCs and de-repression of mRNAs involved in the synthetic phenotype. As a result, the VSMCs transition to the synthetic phenotype associated with atherosclerosis.