Intercellular miRNA Traffic

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Angiogenesis has a fundamental role in tissue homeostasis and likewise has been found to be involved in the pathophysiology of multiple diseases. In a complex interplay, endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and pericytes interact to eventually form new vessels. Most intensely studied in cancer, angiogenesis is regarded as critical for tumor growth and has evolved as a therapeutic target with inhibitors of angiogenesis showing strong antitumor activity. In contrast, modulation of angiogenesis as a therapeutic strategy in cardiovascular disease is less well defined, with both pro- and antiangiogenic principles showing promise in preclinical models. Originating from ECs, this process is tightly controlled by signals that ECs receive and exchange with their environment. These include paracrine signals from the major growth factor families such as the fibroblast and the platelet-derived growth factor families including vascular endothelial growth factor and transforming growth factors. Likewise, important small molecules appear such as nucleotides and lipids, which are increasingly recognized as important paracrine factors within tissues and in angiogenesis signaling. In addition, direct cell-to-cell contacts through gap junctions and direct intercellular signaling through the Notch signaling pathway control EC function during angiogenesis.

Small noncoding RNAs have also been shown to traffic between cells, thereby adding an unexpected level of intercellular gene regulation. In the vascular system, Zernecke et al provided evidence that apoptotic bodies released by endothelial cells contain microRNA-126 (miR-126), which protects tissues and in angiogenesis signaling. In addition, direct cell-to-cell contacts through gap junctions and direct intercellular signaling through the Notch signaling pathway control EC function during angiogenesis.

MiR-143 and miR-145 are distinct in sequence, yet transcribed together as one primary cluster. Being controlled by serum response factor (together with myocardin) and Nkx2.5, expression of miR-143/145 seems to occur primarily in VSMCs throughout various organs. Under certain pathological conditions, however, this cell type preference seems to be partly abandoned, leading to enhanced expression of miR-143/145 in activated ECs and fibroblasts. The joint suppression of specific target mRNAs by miR-143/145 contributes to a contractile phenotype, an interpretation that is supported by mice with genetic deficiency of miR-143/145, which also display reduced vascular tone and blood pressure control.

A question that evolved from these previous findings was whether miR-143/145, which is expressed at high levels in VSMCs, may also represent a signal to neighboring cells.

In their report released in this issue of Circulation Research, Climent et al now provide support for the latter, showing that VSMCs deliver miR-143/145 to ECs through fine intercellular tubes, termed membrane nanotubes or tunneling nanotubes (TNTs). This study originates from the observation that the level of miR-143/145, but not that of its precursor molecule (pri-miR-143/145), rose substantially in ECs when these cells were cultured together with VSMCs. This suggested that VSMCs are the source of mature miR-143/145 in ECs and indeed, the authors find robust increases of pri-miR-143/145 in VSMCs on coculture. Consistent with the VSMC as the primary source for miR-143/145 in this setting, the authors report a loss of miR-143/145 in ECs when miR-143/145 was genetically deleted in VSMCs. From there, the authors delineate a pathway in which secretion of transforming growth factor-β by ECs stimulates miR-143/145 expression in VSMCs and transfer of these microRNAs (miRNA) to ECs (see also scheme in the Figure). In ECs, VSMC-derived miR-143/145 represses hexokinase II and integrin β3 and thereby the angiogenic potential of the recipient cell. Expression of miR-143/145 in ECs could not be achieved by the transfer of conditioned medium or VSMC-derived exosomes and was not sensitive to gap junction uncoupling agents.

Both, exosomes and gap junctions, have previously been reported as potential routes for intercellular transfer of miRNAs. Instead, the authors describe the transfer of miR-143/145 to be sensitive to Lantrunculin A, an inhibitor of TTNs, tiny membrane connections that cultured cells form among each other. High-resolution imaging also allowed the direct visualization of TNTs between ECs and VSMCs and the transport of labeled miRNA molecules within them.

Discovered more than a decade ago to occur in Drosophila and subsequently in various cultured cells and also in vivo, our knowledge about the function of TNTs is still scarce. TNTs have been reported to occur in 2 dimensions (diameter below and above 0.7 μm) with the wider subtype containing microtubules that would permit the transport of organelles such as endosomes and mitochondria. This makes TNTs an intriguing candidate transfer route of...
small RNAs, whose uptake into cells has been linked to the endosomal pathway. Most recently, Thayanithy et al have reported the intercellular transfer of miRNAs through TNTs in ovarian cancer. As illustrated in the Figure, the current study by Climent et al proposes that VSMCs deliver miR-143/145 to ECs, where it regulates several established targets. Although technologically challenging, the authors provide multiple lines of evidence that miR-143/145 in ECs quantitatively derives from VSMCs. Particularly conclusive in this regard is the use of cells that are genetically deficient for miR-143/145, together with quantification of the unprocessed precursor pri-miR-143/145, which the authors find not to be transferred. In the absence of definitive approaches to address the requirement of transport through TNTs, the authors have used the currently available approaches and have also assessed and ruled out other established candidate routes of intercellular transport.

With regard to the latter, in particular, extracellular vesicle-mediated (in particular exosomes) transfer of microRNAs has recently received considerable attention. An early study reported the transfer of miRNAs from tumor cells to recipient cells, which was capable of modulating their phenotype. In the cardiovascular system, several groups have in the meantime demonstrated intercellular transport of miRNAs by exosomes. Interestingly, transfer of miR-143/145 via exosomes also has been described to occur from ECs to VSMCs, that is, in the opposite direction as reported in the current study. How can we reconcile these findings and what might be the advantage of having 2 alternative routes in opposite direction? Although speculative at present, the following aspects may merit consideration: (1) with the concentration of miR-143/145 apparently being considerably higher in VSMCs, this cell type may be expected to deliver part of its miRNA-143/145 content to the cell in direct contact through TNTs. This mode of delivery will be primarily dependent on the extent of TNT formation between donor and recipient cell. (2) In contrast, extracellular vesicle–based exchange may represent a signal in the opposite direction, delivering an antiatherosclerotic signal from the EC to the VSMC compartment. This direction of transport may seem counterintuitive in that a cell type with comparably modest levels of miR-143/145 would be capable of a functionally relevant augmentation of miR-143/145 in VSMCs. Still, several studies have shown selective packaging and enrichment of individual miRNAs in secreted extracellular vesicles. Also, laminar flow induces expression of miR-143/145 in ECs and its levels decline in VSMCs during atherosclerosis thereby facilitating a function for transferred miR-143/145.

The findings by Climent et al are of great interest to those studying the complex interplay of the various cell types involved in angiogenesis as well as to those interested primarily in the function of miRNAs and TNTs. It likewise illustrates that current standard models of isolated cells bear severe limitations, which may partly be overcome through the studies of cocultured cells or intact tissue models.

Although delineating a novel route of intercellular RNA transport in the cardiovascular system, this study naturally opens up several interesting questions: Can the divergent findings on miR-143/145 expression in ECs entirely be explained by the different conditions these were kept (ie, normal cell culture versus laminar flow)? Is the flow-dependent induction of miR-143/145 in endothelial cells accompanied by a parallel increase of pri-miR-143? What are the absolute concentrations of endogenous and transferred miR-143/145 molecules in the coculture setting? As miR-143/145 transfer seems to work across species (see also Figure 1 of Climent et al), RNA sequencing of cocultured cells should be able to address this issue. Are the 2 modes of intercellular transport of miRNA-143/145 always unidirectional or can the direction of transport be changed? What are the molecular mechanism underlying miRNA-transport between cells through either route? How is it regulated? Is there a preference of TNT-mediated transport for certain miRNA species? If yes, what are the underlying mechanisms?

Taken together, the intercellular transfer of microRNA molecules in functionally relevant quantities further increases the complexity of microRNA function. Although this will complicate their analysis, it may provide additional means for future therapeutic intervention.

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References

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