Cardiovascular complications arising as a consequence of vascular disease are on the rise in the world, with an urgent need to develop therapies that can prevent the drivers of atherosclerosis and promote healing of infarcted tissue after an ischemic event. Rupture of culprit atherosclerotic lesions is the main driver of downstream myocardial infarction (MI), accounting for 7 million deaths annually and 23 million individuals living with heart failure worldwide. Accordingly, there has been a tremendous interest in using novel therapies to revascularize ischemic tissue and to repair damaged myocardium after MI. Although there is a clear clinical need to promote collateral vessel growth to facilitate healing and to preserve tissue integrity after an ischemic event, the mechanisms that govern the growth of collateral vessels remain largely unknown. In this issue of Circulation, Pankratz and colleagues use microRNAs (miRNAs) to study the different mechanisms that promote angiogenesis and arteriogenesis and find that microRNA-155 (miR-155) has dual and opposing roles in both processes.

The authors first set out to discover the miRNA-based mechanisms that promote neovascularization. Using a model of femoral artery ligation, they found the expression of 30 miRNAs differentially regulated 7 days after ligation. On the basis of the substantial downregulation of miR-155 during this neovascularization period, they hypothesized that miR-155 antagonizes factors that regulate angiogenesis. Indeed, they followed up by showing that endothelial cells (ECs) devoid of miR-155 (from miR-155−/− mice) have an improved angiogenic capacity compared with wild-type ECs and concluded that miR-155 antagonizes angiogenesis. They demonstrated that miR-155 was able to inhibit angiogenesis through inhibition of AGTR1, a known miR-155 target gene that promotes angiogenesis and inhibits inflammation, in ECs. Using an in vivo Matrigel assay that assesses the sprouting of new capillaries into a basement membrane-like biomaterial, the authors detected an increased number of infiltrating cells in miR-155−/− mice. This led to the hypothesis that inhibiting miR-155 in vivo a model of revascularization would lead to improved blood flow recovery by promoting angiogenesis. However, to their surprise and in contrast to what their in vitro data might have predicted, the absence of miR-155 prevented revascularization after femoral artery ligation. Their investigations revealed that this was attributable to an impairment of infiltration of circulating monocytes into the occluded tissue, which are required for appropriate arteriogenesis. They went on to show that this effect in monocytes was via the miR-155 target gene SOCS1 (suppressor of cytokine signaling-1), which antagonizes Jak/Stat activation of chemokines and cytokines. Therefore, in the absence of miR-155, there are a derepression of SOCS1, a reduction in chemotactic factors, including tumor necrosis factor-α (TNF-α), fewer macrophages found in the intimal space, and thus a reduction in neovascularization. Their findings nicely complement previous studies showing that tumor necrosis factor-α is important for the infiltration of leukocytes and the subsequent proliferation of resident vascular cells during arteriogenesis, which are impaired in mice lacking miR-155. Overall, their findings in vivo demonstrate that the proarteriogenic function of miR-155 outweighs the antiangiogenic function of miR-155, so the net physiological outcome of miR-155 blockade is reduced neovascularization after femoral artery ligation (Figure).

miRNAs have solidified themselves as bona fide regulators of physiological and pathological cardiovascular function. In general terms, miRNAs are 20- to 22-nt single-stranded RNAs that can bind seed sequences in the 3′ untranslated region of target mRNA transcripts with imperfect complementarity, repressing their expression via mRNA degradation or translational repression. The complexity and power of miRNAs come with their ability to repress hundreds of target genes, often regulating an entire network or pathway simultaneously. miR-155 is no exception: It has been shown to target genes that control hematopoietic cell differentiation, B-cell and T-cell responses, and microbial defense, making it a multifaceted immunoregulatory miRNA. Because of its wide expression and multifunctional nature, the present study highlights some important considerations in the study of multifunctional miRNAs like miR-155. First, the importance of studying complex physiological pathways like angiogenesis in vivo cannot be underestimated. In vitro angiogenesis assays are performed in isolated ECs, without the interaction of other factors such as monocytes and lymphocytes and their related secreted factors. Second, it is important to understand the endogenous regulation of miRNAs of interest to fully appreciate their function. For example, factors [VEGF] that influence angiogenesis (vascular endothelial growth factor, basic fibroblast growth factor [bFGF]) downregulate miR-155 expression, and predictably, the absence of miR-155 promotes angiogenesis in vitro. Conversely, factors that promote...
Interestingly, a recent report by Heymans et al. showed that the relative levels of expression of miR-155 in bone marrow–derived macrophages may have a greater influence over miR-155 function in vivo, suggesting that miR-155 expression in monocytes/macrophages has been shown to be activated by inflammation, which agrees with the notion that miR-155 is a proinflammatory miRNA. Although the authors did not fully explore the mechanisms that directly control miR-155 expression, they did find that miR-155 expression in monocytes/macrophages may have a greater influence over miR-155 function in vivo. Interestingly, a recent report by Heymans et al. showed that miR-155 in macrophages drives the inflammatory response to pressure overload in the myocardium, where the absence of proinflammatory miR-155 protects from cardiac hypertrophy and heart failure. Thus, although miR-155 may antagonize angiogenesis in ECs, the expression of miR-155 in macrophages overpowers any EC-driven inhibitory effects on angiogenesis, and hence, macrophage-driven mechanisms dominate in vivo.

The findings of the present study underscore the complexity and differences between angiogenesis and arteriogenesis. By definition, angiogenesis is the sprouting of new capillaries, and it is typically driven by ischemia or hypoxia, which in turn induces the expression of hypoxia-inducible factor-1α, VEGF, and other growth factors that stimulate EC proliferation and permeability. Conversely, arteriogenesis is defined as the growth of collateral vessels from pre-existing arteries after occlusion such as after MI or thrombus. Unlike angiogenesis, arteriogenesis is regulated not by hypoxia but by disturbances in blood flow and induces the expression of chemoattractant factors like MCP-1, recruiting monocytes to the occluded arterial site to promote vascular cell proliferation and expansion. Because Pankratz et al. were initially interested in miR-155 as a regulator of angiogenesis in ECs, they used a Matrigel plug assay, which measures angiogenesis into the hypoxic biomaterial. However, this model simulates only an ischemic environment and is not regulated by changes in shear stress or flow. Conversely, when the authors used a model of complete femoral artery ligation, which drastically changes the flow patterns in the hind limb and simulates primarily arteriogenesis, it enabled them to uncover the opposing roles for miR-155 in arteriogenesis versus angiogenesis. Although the mechanisms that govern angiogenesis are well understood, those that govern arteriogenesis are less so. The revelation that miR-155 acts to control both processes but by very different mechanisms suggests that miR-155 may have been under differential evolutionary pressure in different cell types. As described above, the expression of miR-155 is higher in macrophages than in ECs, and it is possible that miR-155 has been under selective pressure to preserve tissue function in the setting of inflammation or injury, including arterial occlusion. Regardless of its evolutionary origins, it is clear that the proarteriogenic/proinflammatory role of miR-155 dominates in this model; however, it remains to be seen in other models of adaptive neovascularization (ie, within the myocardium after MI) whether miR-155 remains a proarteriogenic miRNA.

miR-155 has been touted as a potential therapeutic target for advanced atherosclerosis owing to its extensively studied mechanisms in the vessel wall. Indeed, miR-155 has been shown to promote lesion development at later, more advanced progression of atherosclerosis, and although it may dampen atherosclerosis at early stages of lesion development, this may be attributable to differential levels of expression of the miR-155 target genes Csfr-1 and Bcl6 at different stages of plaque progression. Thus, therapeutically inhibiting miR-155 for the prevention/treatment of advanced atherosclerosis would be predicted to favor reduced atherosclerotic burden. However, the present study provides a cautionary note in the inhibition of inflammatory factors that promote atherosclerosis: Therapies that inhibit inflammation within the atherosclerotic plaque may concomitantly reduce factors that promote arteriogenesis, providing a therapeutic double-edged sword. If miR-155 is inhibited to reduce atherosclerotic lesion progression, it would be predicted to result in impaired arteriogenesis after an occlusive event such as MI and could promote further injury to the myocardium. Conversely, the delivery of miR-155 replacement therapy to encourage new collateral vessel growth via dampening of SOCS1 and activation of TNF-α could activate inflammation within an atherosclerotic plaque and promote plaque rupture. As the present study of miR-155 reminds us, any therapy that promotes cell mobilization to encourage arteriogenesis for collateral vessel growth may invoke more than just the repair response and may in fact activate monocytes and other inflammatory cells by similar mechanisms in the atherosclerotic plaque. Indeed, the growth factors granulocyte macrophage colony-stimulating factor and granulocyte colony-stimulating factor have been tested in clinical trials to mobilize bone marrow–derived progenitor cells to the site of damaged myocardium to promote collateral vessel growth but have failed clinically because of the potential danger associated with these therapies, including evidence of coronary occlusion and MI after treatment. Like miR-155, these growth factors had been shown in animal models to
promote arteriogenesis, and as mechanistic insights emerge, we now understand that what promotes monocyte and progenitor cell recruitment at one site may do so at the cost of expansion of the atherosclerotic plaque. Although miR-155 may not be an ideal therapeutic target, the identification of others miRNAs that promote neovascularization could lead to novel miRNA-based therapies that are specific to arteriogenesis and do not activate inflammation, avoiding many of the resultant confounders.

The molecular regulators of arteriogenesis continue to emerge, and we can now add macrophage miR-155 to the list. Although therapies that promote the growth of collateral arteries to revascularize and repair the myocardium continue to be elusive, many clinical trials in this area are focused on either delivery of cardiac progenitor cells to the site of injury or delivery of factors that promote the recruitment of progenitor cells from the bone marrow, with the hope that these cells will differentiate into new vascular endothelium or myocardium. Not surprisingly, evidence suggests that miRNAs may be important for the mechanism that promotes the success of cell therapy for myocardial repair. Although a number of miRNA-based therapies are being pursued clinically, the present example of miR-155 highlights the importance of fully elucidating any miRNA mechanisms in the right time, at the right place, and, most important, in the right cell before going down the therapeutic road.

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References
MicroRNA-155 in the Heart: The Right Time at the Right Place in the Right Cell
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