Editorial

Towards Point-of-Care Measurements Using Noncoding RNAs

A Novel Tool to Monitor Aggravation of Advanced Atherosclerotic Lesions

Valentina Paloschi, PhD; Lars Maegdefessel, MD, PhD

Atherosclerosis progressively impairs functional integrity of the vasculature and, depending on which arteries are affected, can lead to a variety of serious complications. Roughly, one quarter of the Western population suffers from the consequences of carotid artery disease, such as a transient ischemic attack or stroke,[1] which includes both neurological and cardiovascular complications. The atherosclerotic plaque, causing the narrowing in carotid arteries, can gradually evolve from a stable to an unstable lesion and ultimately rupture, causing an acute ischemic stroke. Several studies have shown that an early detection (<3 hours) of an acute ischemic stroke, followed by thrombolytic treatment, is associated with reduced mortality and symptomatic intracranial hemorrhage.[2] Consequently, there is a great need for biomarkers that can support clinicians to follow and predict the progression of carotid artery disease toward an unstable, rupture-prone lesion.[3] Capturing high-risk individuals who could benefit from an intensified therapy (including a surgical intervention such as carotid endarterectomies) is of eminent importance.

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Noncoding RNAs can be secreted by cells in a tissue-specific manner, either via passive leakage or via incorporation into subcellular particles.[4] Because of the reported stability and detectability in the peripheral blood, they have recently received a lot of attention as attractive biomarkers.[5] MiRNAs, with a size of ≈20 nucleotides, are the best-studied group of noncoding RNAs in cardiovascular disease development and progression, both from the biomarker, as well as the therapeutic perspective.[6]

A more recent class of noncoding RNAs, circular RNA (circRNA), have emerged as promising biomarkers because of their intrinsic stability. Interestingly—and unlike other noncoding RNA subspecies (long noncoding RNAs in particular)—circRNAs are conserved across species and seem relatively tissue-specific.[7] They have joined 3′-5′ ends, for which a back-splicing variant of expression gives rise to conferring structural stability by lacking the exposed sequence that becomes targetable for endonucleases. However, the mechanisms regulating expression and functionality of circRNAs in the vessel wall remain largely elusive. Boeckel et al[8] have studied the involvement of circRNAs in endothelial cells under hypoxic conditions. They were able to describe a biological function cZNF292, a circRNA, which imposes proangiogenic capabilities in vitro. Other studies have indicated that they can function as suppressors of miRNA activity by acting as sponges.[7] Because of their structural stability in circulation and intriguing tissue specificity, circRNAs are likely to become heavily investigated for their potential as future biomarkers in CVD.[9,10]

In this context, the study by Bazan et al[11] in the current issue of *Circulation: Cardiovascular Genetics* provides an excellent example of how to use an miRNA, miRNA-221, which has previously been reported to be acutely decreased after atherosclerotic plaque rupture,[12] and circRNA-284, which putatively suppresses miRNA-221, for diagnostic biomarker purposes.

The miR-221/222 cluster has early on been indicated as an important mediator in vascular disease development,[13,14] and its regulatory role in smooth muscle cell (SMC) differentiation and proliferation. The cluster, which miR-221 shares with its family member miR-222, has been implicated to become activated via growth factors. Davis et al have shown that miR-221 becomes transcriptionally active on platelet-derived growth factor stimulation, limiting the expression levels of the direct miR-221 targets c-Kit and p27Kip1.[12] Inhibition of p27Kip1 through miR-221 has proven to be an important switch in platelet-derived growth factor–regulated SMC proliferation. Limited expression of c-Kit blocks the transcription of SMC-specific contractile genes via targeting Myocardin, a well established and potent SMC-enriched nuclear coactivator. One can speculate that a decrease of miR-221 expression in the plaque shoulder region might lead to the development of a rupture-prone, unstable lesion in carotid arteries. Interestingly, in the current study by Bazan et al,[11] miRNA-221 in plasma of patients with a plaque rupture (and acute or transient ischemic attack within 5 days before carotid endarterectomy) was repressed, reflecting the observation obtained in concomitant tissue biopsies. Moreover, circulating miR-221 levels are reduced in stroke patients, which suggests that this miRNA could generally serve as a marker for cerebrovascular diseases.[15]

One possible regulation of miR-221 is exerted by the circular RNA-284, which possesses an miR-221 binding site. CircR-284 was also expressed in advanced carotid plaques,

From the Department of Vascular and Endovascular Surgery, Klinikum rechts der Isar, Technical University Munich, Germany (V.P., L.M.); and Department of Medicine, Center for Molecular Medicine, Karolinska Institute, Stockholm, Sweden (L.M.).

Correspondence to Lars Maegdefessel, MD, PhD, Vascular Biology Unit, Department of Vascular and Endovascular Surgery, Klinikum rechts der Isar, Technical University Munich, Ismaninger Strasse 22, 81675 Munich, Germany. E-mail lars.maegdefessel@tum.de

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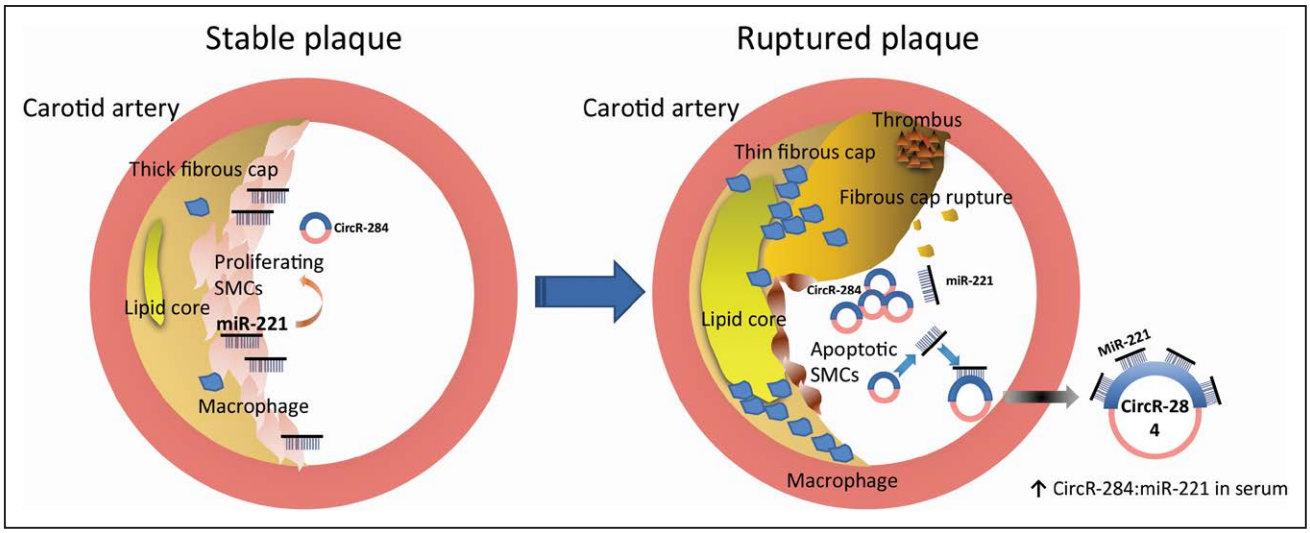

Figure. Bazan et al[11] propose to use the ratio of miR-221:circRNA284, 2 presumably functionally related noncoding RNAs, as a novel biomarker being shed from advanced and vulnerable atherosclerotic carotid artery plaques. SMC indicates smooth muscle cell.

as well as in sera of patients after lesion rupture. An increase in circR-284 could be causatively related with a reduction of miR-221 during plaque rupture, and a decreased ratio of circR-284:miR-221 could indeed be discovered in the urgent/acute patient group. One interesting finding is that the miR-221 expression pattern in serum returns to its normal value 7 days after the event. Moreover, both ncRNAs show an opposite trend of expression in symptomatic and asymptomatic patients, as compared with the urgent group, suggesting that the ratio of circR-284:miR-221 allows for stratification of patients in advanced disease states (Figure).

Bazan et al[11] have proposed to use a pair of functionally related circulating ncRNAs as biomarkers for carotid artery–related ischemic stroke.

Apart from miR-221:circR284, another miRNA has recently been identified for being associated with a higher risk of plaque rupture in carotid arteries. miR-210, which mechanistically targets the tumor suppressor gene adenomatous polyposis coli (*Apc*) was reduced in locally drawn plasma from unstable carotid atherosclerotic plaques compared with stable plaques.[15] In this study by Eken et al,[15] investigating locally drawn, lesion site plasma samples, miR-221 was not significantly different between patients with symptomatic or asymptomatic advanced carotid artery disease. However, only locally collected plasma was profiled, and peripheral miR-221 expression levels were not assessed by the authors.

Obviously, confirmatory large-scale studies destined to replicate the deregulation of miR-221 and its control via circR-284, as well as the secretion of the 2 ncRNAs into serum, would be necessary to validate the potential utilization of the described plasmatic miR-221:circR-284 ratio for biomarker purposes in advanced atherosclerosis. Although the authors suggest that both ncRNAs are expressed in SMCs, the contribution of different cell types within the plaque—and even from different plaques located in other vascular beds—has not been evaluated. Additional approaches, such as laser capture microdissection, could add valuable information on cellular localization of miR-221 and circR-284, enabling researchers to connect the biomarker finding to its relevant biology. Finally, functional studies that aim at repressing circRNA-284 in experimental models of plaque vulnerability and vascular remodeling should be conducted to indicate whether miR-221 increases on circRNA-284 inhibition. This inhibition of the circular RNA and concomitant derepression of miR-221 could have positive effects on plaque stability by enhancing SMC proliferation in the fibrous cap of advanced lesions.

An important consideration to address prognostic power of certain biomarkers is the time point of measurements. The value of the results presented by Bazan et al[11] is connected to the timing of the sample acquisition, which happened shortly after the occurrence of a neurological event. In addition, the comparison of clinical relevance, investigating cases with asymptomatic and symptomatic disease seems intriguing. The amount of time required for disease detection, especially in the case of an acute event, should be short. Point-of-care devices are currently being developed, hopefully enabling us in the near future to measure ncRNAs at the bedside and in emergency settings.[16]

In line with previous studies, Bazan et al[11] suggest that the detection of multiple circulating ncRNAs could be a more powerful diagnostic tool compared with the measurement of only 1 or 2 specific biomarker(s). In addition, the current study indicates that following the expression patterns of tissue-specific noncoding RNAs could enable us to define different levels of disease aggravation, which in consequence allows clinicians to better steer therapeutic interventions.

Disclosures

None.

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