Acute Loss of miR-221 and miR-222 in the Atherosclerotic Plaque Shoulder Accompanies Plaque Rupture

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Background and Purpose—Atherosclerotic plaque vulnerability is accompanied by changes in the molecular and cellular function in the plaque shoulder, including a decrease in vascular smooth muscle cell proliferation. We aimed to determine whether the expression of 3 miRNAs that regulate vascular smooth muscle cell proliferation (miR-145, miR-221, and miR-222) is altered with plaque rupture, suggesting a role in regulating plaque stability.

Methods—miRNAs were measured in the plaque shoulder of carotid plaques obtained from patients undergoing carotid endarterectomy (CEA) for 3 distinct clinical scenarios: (1) patients without previous neurological events but high-grade carotid stenosis (asymptomatic), (2) patients with an acute neurological event within 5 days of the CEA (urgent), and (3) patients undergoing CEA>5 days after a neurological event (symptomatic).

Results—Mean time from plaque rupture event to CEA was 2.4 days in the urgent group. The urgent group exhibited a significant decrease in miR-221 and miR-222 expression in the plaque shoulder, whereas no significant differences were seen in miR-145 across the 3 groups. Regression analysis demonstrated a significant correlation between time from the neurological event to CEA and increasing miR-221 and miR-222, but not miR-145. mRNA encoding p27Kip1, a target of miR-221 and miR-222 that inhibits vascular smooth muscle cell proliferation, was increased in the urgent group.

Conclusions—Atherosclerotic plaque rupture is accompanied by a loss of miR-221 and miR-222 and an increase in p27Kip1 mRNA expression in the plaque shoulder, suggesting an association between these miRNAs and atherosclerotic plaque stability. (Stroke. 2015;46:3285-3287. DOI: 10.1161/STROKEAHA.115.010567.)

Key Words: atherosclerosis carotid stenosis endarterectomy, carotid microRNAs muscle, smooth, vascular

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Results

The urgent group exhibited a significant decrease in miR-221/miR-222, but not in miR-145, when compared with the asymptomatic and symptomatic groups (Figure [A]). Linear regression analysis demonstrated a direct correlation between expression of miR-221/miR-222 and increasing time between carotid plaque rupture/acute neurological symptom onset and the time when CEA occurred ($R^2=0.44; P<0.001$ for miR-221 and $R^2=0.45; P<0.001$; Figure [B]). This correlation is not seen with miR-145 ($R^2=0.004; P=0.85$).

Levels of the mRNA encoding the miR-221/miR-222 target, p27Kip1, were increased acutely post-plaque rupture (urgent group; Figure [C]). Levels of 2 other targets of miR-221/222 that are involved in neovascularization, c-Kit and the signal transducer and activator of transcription 5A, remained unchanged across the groups, suggesting neovascularization is not altered by the changes in expression of miR-221/miR-222 that occur with rupture. These data suggest a role for loss of miR-221/miR-222 inhibition of p27 Kip1 in the thinning of the fibrous cap that promotes plaque instability and rupture.

Discussion

This is the first demonstration that miR-221/miR-222 expression in the plaque shoulder is decreased acutely after plaque rupture. The loss of miR-221/222 was accompanied by an increase in the mRNA encoding its target, p27Kip1. Loss of p27Kip1 through increased miR-221/miR-222 expression results in increased intimal thickening in animal models of vascular injury. VSMCs isolated from advanced atherosclerotic plaques exhibit lower proliferation rates. Similarly, VSMCs isolated from CEA specimens obtained from asymptomatic patients exhibit higher proliferative responses and lower p27 Kip1 levels than those obtained from symptomatic patients. Our data support a role for miR-221/miR-222 in the intimal thickening associated with plaque development, and that loss of miR-221/222 may underlie the reduced VSMC proliferation associated with fibrous cap thinning and plaque rupture.

![Figure](image-url)
in VSMCs, additional studies examining the role of miR-221/miR-222 in regulating plaque neovascularization and macrophage accumulation are, therefore, warranted.

By focusing on carotid plaques obtained from patients undergoing urgent CEAs, we were able to identify modulations in miRNA expression that occur acutely with plaque rupture, but would not be detected in the standard symptomatic patient. Namely, that miR-221/miR-222 expression is reduced in the carotid plaque shoulder at the time of rupture and then returns to prerupture levels within 2 weeks. It is not possible, however, to determine whether the decrease in miR-221/miR-222 occurs before or as a result of plaque rupture. These data highlight the unique value of obtaining samples immediately after an acute neurological event when examining the mechanism underlying plaque rupture and suggest a link between miR-221/miR-222 and cell proliferation in this region.

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Disclosures
None.

References