Platelet microRNAs

Novy Mir* or Mired in Human Complexity?

Anne M. Curtis, Garret A. FitzGerald

A side from their established role in initiating the vascular occlusive events that culminate in myocardial infarction and stroke, platelets are increasingly appreciated for their relevance to inflammation and carcinogenesis. An overview of randomized trials of antiplatelet drugs, mostly aspirin, indicates that they reduce the secondary incidence of important vascular events by ≈20%,1 and provocative albeit inconclusive evidence has emerged, suggestive of their value in cancer.2 Most experience has been gathered with aspirin, which suppresses platelet cyclooxygenase-1–derived thromboxane (Tx) A₂ formation and antagonists of the P2Y₁₂ platelet adenosine diphosphate (ADP) receptor, such as ticlopidine, clopidogrel, and prasugrel.3

A problem with antiplatelet drugs, as with anticoagulant drugs (those targeting elements of the coagulation cascade which are favored for assembly on the membranes of activated platelets), is the segregation of clinical benefit from the risk of bleeding. In the acute clinical setting, such as in patients with ST elevation and myocardial infarction (STEMI), prevention of thrombosis is paramount. Here, potent inhibition of platelet function is the objective, such as can be attained with parenteral administration of inhibitors of the platelet integrin, α₁β₃, bleeding is tolerated, especially if it occurs at a site where it can be controlled, such as at the point of catheter insertion.4 However, during chronic drug administration, such as in the secondary prevention of MI, even nuisance bleeds, which in themselves are not life-threatening, drive down the degree of platelet inhibition that is practical, given their impact on patient compliance. Drugs that block individual pathways of platelet activation rather than a final step, such as fibrinogen binding of α₁β₃, have found favor in this setting. However, even low dose aspirin roughly doubles the low incidence of serious gastrointestinal bleeds compared with placebo, and the incidence of this adverse effect may rise sharply with age.5

When aspirin and clopidogrel are combined, the incidence of nuisance bleeds can be as high as 30%.6

So how can we predict efficacy and the risk of bleeding in patients to whom we wish to give antiplatelet drugs? Not easily. Trivial assays permit detection of dose-dependent suppression of platelet capacity to generate TxA₃ or of TxA₃ receptor–dependent platelet aggregation ex vivo. However, neither approach measures directly cyclooxygenase-1 acetylation by aspirin nor do they reflect platelet activation in vivo. Indeed, aggregation assays ex vivo may be paradoxically depressed in syndromes of platelet activation,7 perhaps reflecting selective harvesting for the assay after activation of the platelets most sensitive to agonists in vivo. Although these comments also extend to the use of ADP-induced platelet activation in patients taking P2Y₁₂ antagonists, here the most common approach is to measure vasodilator-stimulated protein phosphorylation, which regulates actin filament growth in platelets. The induction of vasodilator-stimulated protein phosphorylation by activators of guanylate or adenylylcyclase is suppressed by ADP-induced activation of P2Y₁₂ on platelets. Blockade of P2Y₁₂ prevents the suppression by ADP of vasodilator-stimulated protein phosphorylation, and this phenomenon has been developed into a platelet reactivity index of P2Y₁₂ blockade. Gene variation may result in resistance to platelet inhibition by P2Y₁₂ antagonists.1 The vasodilator-stimulated protein phosphorylation assay is unaffected by aspirin treatment, detects the effects of all ADP antagonists, and has been claimed to discriminate between subjects using treatment who go on to experience thrombotic events and those who do not.8 By contrast, claims that tests of platelet aggregation, serum TxB₂, or urinary Tx metabolites can detect a syndrome of aspirin resistance seem, at best, questionable.9 Finally, prolongation of the bleeding time by antiplatelet drugs has proven to be a quantitatively unreliable predictor of bleeding risk. In sum, presently available assays indirectly reflect the mechanism of antiplatelet drug action and have not been established conclusively as indicators of either clinical benefit or risk.

In the present issue of Circulation Research, Willett et al10 suggest that platelet noncoding regulatory microRNAs (miRs) might reflect quantitatively platelet activation in vivo and, as such, might represent biomarkers of cardiovascular risk and a means by which to titrate to efficacy antiplatelet drugs. miRs are ≈20 to 25 nucleotides long and regulate gene expression through targeting and degradation of mRNA or inhibition of translation. miRs act to fine tune biological processes and appear to have a role in all aspects of cellular biology. Because each miR is predicted to bind to ≈200 genes, often, if not always, functionally integrated cassettes, it is unsurprising that miRs are thought to regulate >30% of the human protein coding genome11: >2000 mature human miRs are listed in

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*Novy Mir (New World), a famous Russian literary magazine.

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Aside from their relevance as regulators of gene expression, miRs are remarkably stable in serum and plasma and have been touted as prognostic indicators of cancer and cardiovascular risk. The present study was prompted by the results of an earlier study of 820 participants in a population-based survey designed to identify predictors of MI. Forty-seven MIs occurred over 10 years of follow up, and plasma miR-126 was positively associated with incident MI, whereas miR-197 and miR-223 were inversely related to disease risk. No relationships with incident stroke were observed.

In a subsequent intervention part of this study, 11 healthy volunteers underwent limb ischemia-reperfusion generated by thigh cuff inflation, and plasma miRs were measured over the next 7 days. The investigators focused again on miR-223, -126, and -197 because they were all upregulated early and in a sustained fashion after the period of ischemia-reperfusion (despite their divergent relationship to incident MI); yet only miR-126 reached statistical significance. All could derive from platelets and platelet microparticles, small vesicles that bud from activated platelets, and have been implicated in regulation of endothelial gene expression.

Here Williet et al compare a panel of 337 miRs in platelet-rich plasma, platelet microparticles, and platelet-poor plasma from only 3 volunteers and report that miR-223 was the most differentially expressed and also provided the greatest degree of discrimination between serum and platelet-poor plasma in 19 diabetic subjects. They then measured a panel of 92 miRs in platelet-poor plasma at weekly intervals in an open, uncontrolled evaluation of dosing with prasugrel 10 mg/d with aspirin 75 mg/d and 300 mg/d added in successive weeks in 9 healthy male volunteers and found that among the 15 miRs depressed were miR-223, -126, and -24. Finally, in 12 patients with documented carotid atherosclerosis on 75 mg/d aspirin, the addition of either diprydamole or clopidogrel depressed some (including miR-126 and miR-223) but not all of the miRs depressed by antiplatelet drugs in the volunteer study.

What to make of all this? The authors suggest that their work indicates the potential use of miRs as predictors of cardiovascular risk and more pertinent to the present study, in vivo indices of platelet activation that may have practical value as a guide to the efficacy of therapy. However, perhaps more evidence is needed before we can accept these suggestions. For instance, why are these platelet-derived miRs differentially related to cardiovascular risk? If miR-223 is suppressed by antiplatelet drugs, how do we interpret its inverse relationship to incident MI? In particular, we need to know more before we can assess the value of plasma miRs as useful indicators of platelet activation in vivo. Even in the context of an exploratory study, the sample sizes in some of the experiments reported in the present study are very small. Furthermore, the study designs are unblinded and not randomized and the results from the 2 studies are often discordant across phenotypes and experimental paradigms. Additional notes of caution include our ignorance of the influence of such variables as gender, time of sampling, patient age, diet, or concomitant anti-inflammatory or hypolipidemic medications on levels of circulating miRs.

In subsequent, more rigorously designed studies, it would also be helpful to have corresponding information on the levels of platelet, endothelial, and leucocyte microparticles and the corresponding abundance of miRs. For example, if one focuses on the most promising miR in the current study, miR-223, does it impress because it is most stably expressed among miRs in platelet microparticles and thus merely reflects their shedding of platelet microparticles? How specific would it be as a platelet signature? We know that miR223 has also been detected in microparticles shed by macrophages from which it can be trafficked to endothelial cells to influence function.

Reminding us that platelets contribute substantially to miRs in plasma and that antiplatelet drug consumption may confound interpretation of concentrations in clinical trials is valuable. Furthermore, Williet et al make a provocative suggestion about the potential utility of platelet-derived miRs as quantitative indices of either platelet activation or cardiovascular risk. However, there is little for now to suggest that measuring miRs in plasma will provide information of incremental value beyond current approaches and none to indicate that they might help segregate clinical benefit from risk—perhaps the biggest challenge to the use of antiplatelet and anticoagulant drugs.

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