Platelet microRNAs and vascular injury

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Vascular smooth muscle cell (VSMC) phenotype switching from a contractile state to a synthetic phenotype has been implicated in intimal remodeling during vascular injury. While multiple studies have focused on dedifferentiation of VSMCs, prevention of VSMC-mediated excessive repair remains poorly understood. In this issue of the *JCI*, Zeng et al. identified a mechanism by which platelet-derived microRNA-223 (miRNA-223) reverses VSMC dedifferentiation. The authors show that suppression of proliferation occurs after platelet internalization by VSMCs. Moreover, they demonstrate that miRNA-223 inhibits dedifferentiation and intimal hyperplasia in diabetic mice by decreasing PDGFRβ expression in VSMCs. Together, these results identify platelet-derived miRNA-223 as a potential therapeutic target in vascular injury.

Platelets and vascular injury

It is well established that following vascular injury, platelet surface receptors bind to the exposed subendothelial extracellular matrix, which leads to platelet activation and the release of the content of their granules, causing platelet aggregation and thrombus formation (1). Recent studies, however, suggest a much more complex role for platelets in vascular injury (2); for instance, they are thought to mediate inflammatory responses in adjacent tissues by supplying micro RNAs (2). Platelets, while being anucleate cells with relatively low levels of protein synthesis, harbor an extremely abundant and diverse array of microRNAs (3). They also contain all the cytoplasmic protein components of the microRNA pathway (3–5); however, whether or not active processing of pre-microRNA takes place within platelets remains unclear (2). To prevent microRNA degradation in the bloodstream, platelet-derived microRNAs are packaged into microparticles that are released upon platelet activation (2) to be then delivered to the recipient cells (6). In the current issue of the *JCI*, Zeng et al. provide the first report of microRNA transfer between platelets and vascular smooth muscle cells (VSMCs) and describe another putative mechanism of microRNA delivery — through the direct internalization of platelets by VSMCs (7) (Figure 1).

Platelet microRNAs

While numerous microRNAs have been identified in platelets (8), the target and function are known for only a handful of them (2). Since most of these microRNAs can be detected in the bloodstream, they have been considered mainly as potential biomarkers (9). For example, higher levels of miRNA-126 were associated with improved cardiovascular outcomes in patients with stable coronary artery disease (10), an effect that is thought to be mediated through suppression of VCAM-1 expression on the endothelium, diminished leukocyte adhesion, and reduced inflammation (11). Some of the miRNAs that are found in platelets exhibit differential effects in endothelial cells and VSMCs. For example, miRNA-22, one of the miRNAs increased in supernatants of platelets after aggregation, can be taken up by endothelial cells and results in decreased ICAM-1 expression (12). In contrast to this anti-inflammatory effect on the endothelium, in VSMCs, miRNA-22 (although not from platelets) has been shown to promote phenotypic switching and neointima formation (13). miRNA-143 and -145, in contrast, induce differentiation and repress proliferation of VSMCs (14, 15). Zeng et al., however, provide evidence that at least some of the miRNA-143 and -145 in VSMCs during vascular injury is likely to originate from activated platelets. These findings indicate the complexity of miRNA-mediated intercellular signaling, which is dependent on the exact miRNA, the delivery mechanism, and the recipient cell. Further investigation of the distinct roles of platelet-derived miRNAs in various tissues, and even cell types within a particular tissue, is thus warranted. This knowledge can provide insight into therapeutic targets for such vascular processes as micro- and macrovascular complications of diabetes (16) and re-endothelialization and VSMC proliferation after stent placement (17).

miRNA-223

While miRNA-143 and -145 are expressed in both platelets and VSMCs, miRNA-223 expression is restricted to hematopoietic cells and is one of the most abundant miRNAs in platelets (9). It is also the only miRNA that has been linked to P2Y12 expression and reactivity to antiplatelet agents (9). Patients with diabetes were found to have lower levels of miRNA-223, resulting in higher expression of P2Y12 receptor and P-selectin, thus contributing to platelet hyperactivation (18). Moreover, lower levels of miRNA-223 were associated with a higher incidence of stroke (19) and increased mortality (20).

Although the aforementioned effects of miRNA-223 on platelet function have been studied quite extensively, knowledge of its role in other cells remains limited. Interestingly, freshly isolated endothelial cells have high levels of miRNA-223, which are lost early during culture (21). It was proposed that in vivo microRNA-223 levels in endothelial cells are maintained via microparticle-dependent transfer from platelets (6). Such

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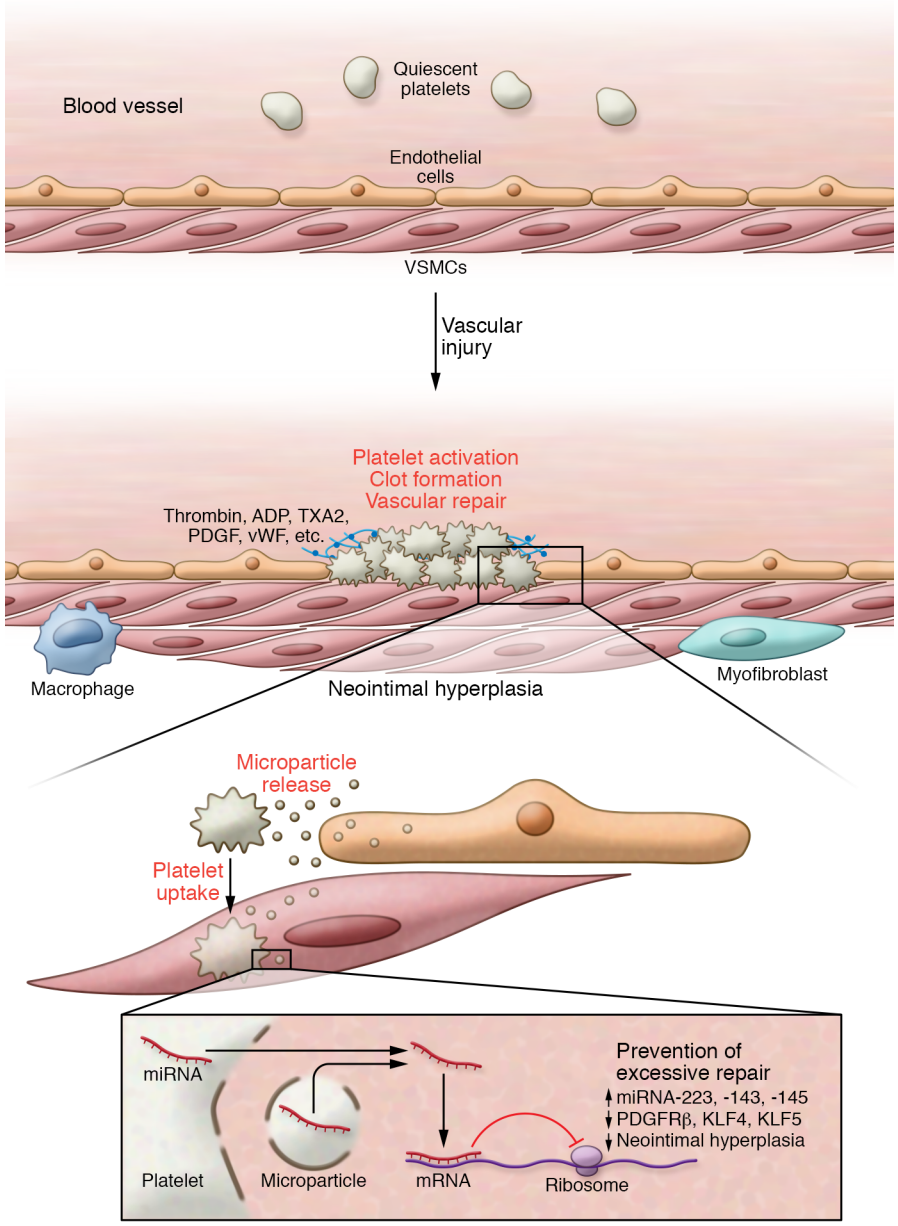

Figure 1. Summary of the possible mechanisms for platelet-derived miRNA transfer during vascular injury. With intact endothelium, platelets are quiescent. When the endothelial barrier is compromised, platelets are activated, form a clot, and release various factors promoting vessel repair. They also release microparticles (MPs) containing miRNAs that are taken up by endothelial cells (and possibly other cells) and exhibit various effects on protein expression in recipient cells. Next, the platelets are taken up by VSMCs and lysed, releasing miRNAs into cytoplasm of VSMCs. In particular, miRNA-223, -143, and -145 released in VSMC cytoplasm decrease expression of PDGFRβ, KLF5, and KLF4, thereby suppressing VSMC dedifferentiation and excessive repair.

transfer had not been shown for VSMCs, however, until, in the current issue of the *JCI*, Zeng et al. demonstrated an important role for platelet-derived miRNA-223 in VSMC differentiation. The authors also showed that delivering the AgomiRNA-223 complex in miRNA-223–deficient mice (as a result of either genetic modifications or diabetes) can abrogate intimal hyperplasia after injury. This makes miRNA-223 a promising therapeutic modality for prevention of neointima formation after vascular injury, particularly in diabetes.

Transfer of platelet miRNAs and their effect on vascular injury

The findings by Zeng et al. add to our understanding of the regulation of intimal remodeling after vascular injury and should have a significant impact in the field. The use of transgenic mice with fluorescent lineage tracers allowed for an elegant demonstration of platelet uptake by VSMCs after vascular injury in vivo. Moreover, the authors identified microRNA-143, -145, and -223 as critical in driving differentiation of VSMCs after platelet internalization. They further pinpointed KLF4, KLF5, and PDGFRβ as targets of these microRNAs. Finally, for the first time, they provided data supporting the role of miRNA-223 in intimal hyperplasia in a diabetes model, making it a compelling therapeutic target for this common condition.

Nevertheless, several important questions remain to be answered.

Zeng et al. propose a hitherto unexplored mechanism of miRNA delivery to VSMCs — by direct internalization of platelets. They provide electron micrographs of the internalized platelets and demonstrate significantly higher levels of platelet-specific miRNA-223 in VSMCs after incubation with activated platelets compared with platelet-derived microparticles. While these results are intriguing and seemingly support miRNA transfer via platelet internalization, it is also possible that both microparticle-dependent miRNA transfer and direct internalization of platelets take place upon injury. Confocal images of cocultured platelets and VSMCs from this study reveal internalized platelets along with platelets that appear to be adherent to the VSMCs. While this may represent platelets in various stages of internalization, it also allows for the possibility that some platelets supply miRNAs via microparticles. Additionally, with the described design of the in vivo experiments, one cannot distinguish between platelet uptake by VSMCs and other cell types (e.g., fibroblasts, macrophages). This is particularly important given the recent discovery that during the response to vascular injury, VSMCs can assume a macrophage-like phenotype, a transformation that may explain platelet uptake by these cells (22). More detailed studies with lineage-specific tracers or staining are needed in order to identify the proportion of platelets internalized by VSMCs. The exact cellular machinery involved in platelet internalization also remains to be described.

Of interest, while platelet uptake by VSMCs and an increase in miRNA-223 levels coincide within hours of incubation with activated platelets, the highest levels of miRNA-143 and -145 in VSMCs were observed much later, at 24 hours. One of the possible explanations for this observation is miRNA synthesis within VSMCs themselves. Further work is needed, however, to elucidate the exact mechanism of this delayed signaling potentially responsible for continuous prevention of neointima formation.

Finally, the authors demonstrate an effect of high glucose on the levels of selected miRNAs in platelets. It is important to know, however, whether other microRNAs as well as mRNAs are affected. Currently, the data on this subject remain controversial (23) and will require further investigation. Unraveling the mechanism by which glucose exhibits this effect may also reveal additional therapeutic targets for prevention of vascular complications in diabetes.

Zeng et al. complete their study by proposing a potential role for the described pathways in other vascular pathologies, such as atherosclerosis. While this concept is intriguing, further work is needed to confirm this hypothesis. Still, the findings presented in this article have uncovered a potentially targetable pathway in a field of utmost public health significance.

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