Platelets deliver small packages of genetic function

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In this issue of Blood, Laffont et al have identified a type of delivery system by platelets in the form of platelet microparticles delivering functional microRNA to endothelial cells. The delivery, it appears, may be a relatively novel process of regulation of gene expression in endothelial cells and potentially other nucleated cells of the body.1

Platelets are anucleate fragments with a diameter of 1 to 4 μm that are released by bone marrow megakaryocytes into the circulation and are thought to be primarily responsible for the maintenance of vascular integrity and hemostasis. There is a growing body of evidence to suggest that platelets also contain a vast array of proteins and genetic material that may have functions beyond hemostasis. For example, human platelets contain an abundant and diverse array of microRNAs that may be involved in regulating messenger RNAs (mRNAs), protein synthesis, and reactivity. MicroRNAs are 19- to 24-nucleotide noncoding RNAs that are involved in sequence-specific, post-transcriptional regulation of gene expression.2-4 They are generated by a ribonuclease called Dicer that processes microRNA precursors into microRNAs that are then incorporated into microRNA effector complexes containing Argonaute 2 (Ago2) protein.5 Their biological role appears to be linked to their ability to mediate sequence-specific regulation/repression of mRNA translation by recognizing and binding to specific sites mainly located in the 3' untranslated region. Although the majority of microRNAs are found intracellularly, several have been identified outside of cells in various body fluids where they may represent a class of stable biomarkers for disease.

Activated platelets also release microparticles (MPs) that are small extracellular vesicles ranging from 0.1 to 1 μm in diameter and are shed from the cytoplasmic membrane. Platelet MPs are the most abundant cell-derived MP subtype in the circulation and have been shown to be associated with inflammatory diseases such as arthritis.6 Platelet MPs are also known to contain a broad variety of cytoplasmic components, including proteins, DNA, and RNA. It appears that these small lipid vesicles may thus act as intercellular carriers and deliver bioactive proteins, RNA and microRNA.
to recipient role as a source of genetic information that can be transferred from one cell type to another across the circulatory system, including the endothelial cells lining the inner surface of the vasculature.

In this issue, Laffont et al. have further elucidated the role of how platelet-derived cargo can be transferred (see figure). They found that activated platelets release microRNA miR-223 preferentially through MPs, and these MPs can be internalized by endothelial cells. What is intriguing is that the platelet MP-derived Ago2•microRNA complexes are functional and can regulate endogenous gene expression in recipient endothelial cells. First, they showed that MPs could be released from thrombin-activated platelets and had diameters of 100 to 400 nm and expressed CD41a. What was more striking was that compared with spontaneous released MPs from resting platelets, the thrombin-induced platelet MP preferentially contained one of the most abundant platelet microRNAs termed miR-223 complexed with Ago2. They chose to study this particular microRNA, as it is abundantly found in platelets, but it is rather scarce in cells such as endothelial cells. Using confocal fluorescence microscopy, they then went on to show that the Ago2•miR223 complex-laden platelet MPs could be efficiently internalized by human umbilical vein endothelial cells (HUVECs), and they could persist in the cytoplasm for up to at least 48 hours. This increase and persistence of elevated miR-223 levels in the HUVECs suggested that there may be a time window necessary for the platelet-derived Ago2•miR-223 to regulate HUVEC gene expression. In fact, when they transiently transfected the HUVEC with the Rluc reporter gene construct that was placed under the control of a miR-223 binding site, there was a significant decrease in HUVEC reporter gene activity induced by coinubcation with the MPs. Then they searched for potential endothelial mRNA targets of platelet miR-223, and identified 2 mRNA candidates, FBXW7 and EFNA1, that contain 4 and 1 conserved miR-223 binding sites in their 3’ untranslated region, respectively. They observed a significant downregulation of endogenous FBXW7 and EFNA1 mRNA levels within 6 hours of exposure to platelet MPs and a significant reduction in FBXW7 and EFNA1 protein levels by 18 hours and 96 hours, respectively.

Thus, it appears that activated platelet-derived MPs can act as carriers and mediate the transfer of proteins and RNAs between cells. Although this has been demonstrated by others, the current report highlights the relative complexity, efficiency, and functional importance of intercellular communications across the circulatory system. Perhaps more importantly, considering the diversity of platelet microRNA sequences, as well as the number of cell types potentially capable of exchanging genetic materials, it is possible that MPs released by activated platelets may communicate this information and modulate or adapt the responsiveness of the vasculature accordingly. It will be interesting to determine whether activated platelet-derived MPs are able to transfer functional genetic material to other cell types within the circulation or spleen (eg, macrophages that can act as both pro-inflammatory cells and antigen-presenting cells and modulate their function in different inflammatory states).

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REFERENCES


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HIT: nucleic acid masquerading as heparin

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In this issue of Blood, Jaax and colleagues show that heparin-PF4 antibodies cross-reacted with nucleic acid (NA)–PF4 complexes and induced platelet activation, suggesting that NA-PF4 can potentially cause a heparin-induced thrombocytopenia (HIT)–like prothrombotic disorder.

HIT is a serious prothrombotic complication of heparin treatment. Approximately 50% of patients who develop HIT suffer thrombosis that is often severe and extensive. Even with treatment using a nonheparin anticoagulant (such as argatroban), death and leg amputation (due to limb gangrene) still occur in 23% and 15% of patients, respectively. HIT is mediated by immunoglobulin G (IgG) heparin-PF4 antibodies. Heparin-PF4-IgG complexes bind and cross-link platelet FcγRIIA receptors inducing platelet activation, granule and microparticle release, platelet aggregation, and thrombus formation. For many years, clinicians have been puzzled by several unexplained observations (see figure). First, HIT occurs more frequently in patients with tissue damage, which can occur after a major surgery, major trauma, or severe infection. Second, a HIT-like thrombotic disorder (termed “spontaneous HIT”) develops in
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