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PLATELETS AND THROMBOPOIESIS

Comment on Volz et al, page 2696

GPVI inhibitor as antitumor gateway drug

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In this issue of *Blood*, Volz et al establish a potential antitumor strategy by exploiting the selective requirement for platelets to maintain vascular integrity within the tumor microenvironment.[1] Their work demonstrates, for the first time, that functional inhibition of platelet-specific surface receptor glycoprotein (GP) VI, using F(ab')$_2$ fragments to avoid platelet clearance, increases intratumoral hemorrhage and concomitant tumor cell apoptosis, as well as enhanced accumulation of chemotherapeutic drugs. These effects work additively to inhibit tumor growth, achieving results similar to those achieved by platelet depletion.[2,3]

Among platelet receptors, GPVI possesses the rare property of being nonessential for hemostasis, but its loss or blockade prevents arterial thrombosis, making GPVI an attractive target. Indeed, Revacept, a soluble dimeric GPVI fusion protein, is currently in phase 2 trials as an antithrombotic therapy.[4] Platelets are small circulating anucleate cell fragments that are essential for hemostasis, but platelets are increasingly recognized as mediators of a broad range of hematologic functions. Platelets have been shown to safeguard the integrity of developing and dysfunctional vessels under inflammatory conditions. GPVI was recently established as an essential mediator of vascular integrity in inflammatory settings.[5] The tumor microenvironment is one such setting. The study by Volz et al is the first to investigate this function of GPVI in solid tumors. Using both orthotopic and heterotopic models of tumor implantation in mice, they demonstrate increased intratumoral hemorrhage with either GPVI depletion in the host, or acute GPVI inhibition using an F(ab')$_2$ fragment of JAQ1, an antibody that blocks the major collagen binding site on murine GPVI. The results achieved are similar to those obtained with acute platelet depletion.[2,6] The treatments directed against GPVI also increased the accumulation of chemotherapeutic drugs in the tumors. With anticancer drugs given every 4 days, the authors observed additive effects of GPVI inhibition or platelet depletion on tumor growth suppression. This provides proof of concept for combined GPVI targeting with chemotherapeutic drugs as a potentially effective antitumor approach targeting specific platelet functions but with minimal bleeding complications.

Unlike most current antiplatelet antibodies, the JAQ1 F(ab')$_2$ fragment does not lead to platelet clearance. Thus, the ability of JAQ1 F(ab')$_2$ to induce intratumoral hemorrhage can be attributed to molecular blockade of GPVI on circulating platelets, although contributions of plasma GPVI shed from platelets cannot be ruled out. This in itself is a striking result, because it indicates that GPVI exposure is the principal mediator of platelet-dependent vascular integrity. Of particular note is that mechanisms of GPVI-dependent vascular integrity in inflammation appear to vary depending upon the extent of vascular damage and the underlying context.[7] In the case of small breaks in the endothelial barrier exposing subendothelial collagen and laminin, single platelets can plug the leak via GPVI engagement in many inflammatory settings. This may be the case in dysfunctional tumor vasculature.[8] Indeed, Volz et al were able to reproduce the hemorrhage and tumor growth inhibition of JAQ1 F(ab')$_2$ using soluble dimeric GPVI-Fc fusion protein, which competes for platelet-collagen binding, providing further support for this mechanism in the solid tumor models. However, GPVI inhibition caused massive intratumoral hemorrhage beyond what might be anticipated by single platelet-sized gaps in endothelium. Earlier studies demonstrated that infiltrating leukocytes are the major drivers of platelet-dependent intratumoral hemorrhage.[9] One possible explanation for the increased intratumoral hemorrhage in GPVI-blocked mice could involve multiple steps. GPVI is required initially to establish single platelet plugs via anchorage and spreading on subendothelial matrix. In the absence or blockade of GPVI, inflammatory cells, principally neutrophils, infiltrate and induce further vascular damage, thereby increasing the extent of hemorrhage, as observed by Volz et al. Neutrophil recruitment to the tumor microenvironment was not altered by GPVI inhibition, supporting a role for GPVI in either preventing or possibly repairing vascular damage induced from neutrophils. However, neutrophil depletion did not fully prevent hemorrhage by GPVI blockade, indicating contributions from other factors.

Tumor-associated macrophages, other inflammatory cells, as well as plasma GPVI, may also play important roles. Moreover, platelet-derived permeability factors, such as serotonin, vascular endothelial growth factor, and angiopoietin-1, have not yet been investigated in this context.[2] Dynamic studies of platelet and leukocyte interactions with the vessel wall in tumor models, coupled with analysis of soluble factors, will be essential to elucidating the cellular and molecular basis for intratumoral hemorrhage induced by GPVI blockade.

Platelets influence solid cancer progression through many mechanisms, and new roles for platelets are continually emerging. A striking outcome of the study from Volz et al is the provocative notion that GPVI inhibition could have anticancer clinical utility by taking advantage of several of these mechanisms. First, GPVI inhibition caused tumor cell apoptosis and reduced growth of solid tumors by selectively driving intratumoral hemorrhage. Second, increased vascular permeability selectively in tumors potentiated intratumoral accumulation of commonly used cancer chemotherapeutics, both paclitaxel and liposomal doxorubicin. Third, GPVI depletion has been shown to limit metastatic dissemination in some ectopic tumor models in mice, although metastasis was not tested in this study.[10] Although it is established that GPVI modulation blocks thrombosis but is permissive for hemostasis, GPVI blockade also appears to have no effect on integrity of intact vessels. Together, these properties of GPVI inhibition support an attractive multifaceted approach to multistage cancer treatment, with potentially limited side effects compared with current antiplatelet therapeutic approaches. However, inflammation may present a substantial obstacle, because GPVI inhibition may also drive hemorrhagic responses at inflammatory sites other than the targeted tumor tissue. Hence, although GPVI blockade in combination with chemotherapeutic regimens may help deliver the triple-play to knock out malignancy, underlying inflammation may also be targeted with potentially dangerous results. It will be critical to determine whether effects of GPVI targeting in solid tumors reflect a common mechanism of increased hemorrhage at inflammatory sites, or if those effects are unique to the tumor microenvironment.

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VASCULAR BIOLOGY

Comment on Streetley et al, page 2707

WPBs and α-granules: more and more look-alike?

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In this issue of *Blood*, Streetley et al have used live-cell imaging and high-resolution cryo–electron microscopy tomography to identify CD63⁺ luminal membrane vesicles in Weibel-Palade bodies (WPBs) in human umbilical vein endothelial cells (HUVECs) and microvascular endothelial cells. In response to an increase in intracellular calcium or cyclic adenosine monophosphate, these membranes are released as so-called exosomes in a fashion similar to that described for platelets and other cells. This is the first report of the presence of intraluminal vesicles (ILVs) in WPBs and the regulated release of exosomes from vasculature endothelial cells.[1]

WPBs in endothelial cells belong to the group of lysosome-related organelles (LROs), a heterogeneous group of organelles that share features with lysosomes and secretory granules.[2] LROs acquire cargo and membrane components both from the biosynthetic pathway and the endo-lysosomal system. Formation of WPBs starts at the *trans*-Golgi network (TGN) and is mainly driven by the assembly of von Willebrand factor (VWF) multimers into tubules that shape the organelle into an elongated structure. Other cargo molecules such as cytokines and the membrane protein P-selectin are included during WPB maturation. Additional components such as the tetraspanin protein CD63 (also called lysosomal-associated membrane protein 3 [LAMP3]) become incorporated at a later stage.[3] CD63 shares with LAMP1 and LAMP2 a cytoplasmic Gly-Tyr motif, which serves as a lysosomal-targeting signal.[4] CD63 is a well-established component of the late endosomal and lysosomal system in many cells. Although CD63 is found in WPBs, the vast majority of CD63 is present within the complex network of internal membranes characteristic of late endosomes. CD63 in WPBs presumably derives from these endocytic compartments.[3] Indeed, as shown by Streetley et al in the present study,