Thrombus stability on the vessel wall

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In this issue of Blood, Ono and colleagues use sophisticated imaging technology to identify a fibrin-independent platelet contractile mechanism regulating primary hemostasis and thrombus growth.

S haughnessy and colleagues were the first to describe a potential role for the canonical Wnt inhibitor, dickkopf-1 (DKK1), 5 years ago when they reported abnormally high levels of the secreted glycoprotein in the blood of individuals newly diagnosed with multiple myeloma (MM). Of note was a correlation between the levels of DKK1 and the extent of osteolytic bone lesion (OBL) formation, a hallmark characteristic of the disease. In about 80% of MM patients, OBLs occur throughout the skeleton, causing intractable bone pain and pathological fractures. The MM cells flourish and divide within the cytokine–enriched cavi-
ties of the OBLs, perpetuating a destructive cycle that is the major cause of morbidity. Historically, the treatment of OBLs has focused on inhibition of osteoclastogenesis, but even when osteoclast activity is controlled, the lesions never repair, suggesting that MM cells also disrupt osteoblastogenesis. There is evidence that DKK1 affects OBL pathogenesis through inhibition of Wnt-mediated differ-
tiation of osteoprogenitors in the bone mar-
row, and one would predict that this could lead to attenuation of the repair of bone tissue. Indeed, Yaccoby, Shaughnessy, and col-
leagues have demonstrated that administration of DKK1–sequestering antibodies can prevent inhibition of osteogenesis in a model of MM and can reduce the formation of OBLs. In the current study, Qiang and colleagues have expanded our knowledge of the mecha-
nism of DKK1 action by demonstrating that, in addition to inhibition of osteoblastogenesis, DKK1 causes a drop in osteoprotegerin (OPG) expression and an increase in receptor activator of nuclear factor κB ligand (RANKL) output by preexisting osteoblasts. This observation is significant because OPG is the scavenger receptor for the osteoclastogenic protein, RANKL; high ratios of locally se-
creted RANKL-to-OPG levels result in up-
regulated osteoclast activity and bone destruc-
tion. Therefore, MM-derived DKK1, in addition to its ability to inhibit osteoblast differ-
entiation, is osteoclastogenic through disrup-
tion of RANKL/OPG homeostasis.

In a series of in vitro assays, the investigators demonstrate that addition of recombinant Wnt3a to various cultured osteoblast cell lines resulted in up-regulation of OPG output and reduction of RANKL expression. Addition of recombinant DKK1 attenuated the effect by reducing OPG output. This observation was confirmed by DKK1 overexpression and RNAi-
mediated silencing in the osteoblast lines. Signifi-
cantly, DKK1 present in MM cell–
conditioned media had similar effects in increasing the ratio of RANKL-to-OPG expression in osteoblasts, but when immunosequestered from the media, there was recovery of OPG expression and a concomitant reduction in the ratio of RANKL-to-OPG output. In support of these findings, Gunn et al reported a similar up-
regulation of OPG when Wnt signaling in pri-
mary human osteoprogenitor cells was up-
regulated by treatment with a glycogen-
synthetase-3B-inhibitor. If the cell lines used in the study faithfully recapitulate osteoblast physiology, these observations predict a future strategy for the treatment of MOBLS through coadministration of DKK1–blocking antibodies and OPG.

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REFERENCES


P latelet adhesion, activation, and aggrega-
tion, together with thrombin generation and fibrin formation, are essential steps in the formation of a platelet plug and the arrest of bleeding. Important gaps in our knowledge exist regarding the ways by which thrombus size and stability are regulated in flowing blood. Ono and colleagues have used fluores-
cent markers and an in vitro perfusion system to follow collagen–dependent platelet deposition and fibrinogen incorporation into the building thrombus. Real-time analysis and confocal microscopy reveal a dynamic process with a previously unrecognized contractile activity that assures the tight packing of aggre-
gated platelets. With calcium dependence and kinetics different from those found in fibrin clot retraction, reduced thrombus volume was prevented by blocking nonmuscle myosin heavy chain-IIA (myosin-IIA) activity with blebbistatin and by Rho kinase antagonism. Blocking the tight packing of platelets resulted in the formation of less stable thrombi. Using a novel intravital mouse model for assessing primary hemostasis and FeCl3-induced vascular injury, the investigators confirmed that contractile activity is critical for maintaining the integrity of the hemostatic plug in vivo. Local injection of blebbistatin or a Rho kinase antagonist into the microcirculation resulted not only in a loss of tight packing between platelets in the outer layers of formed thrombi, but also in progressive embolization from the thrombus surface—implying that the process is reversible. Studies on PAR+/- mice con-
firmed that thrombin, while necessary for opt-
imal thrombus formation, was not essential for contractile activity. Not only does this study open up new avenues for antithrombotic therapy; it also provides insights into the pathophysiology of MYH9–related platelet disorders that principally affect myosin–IIA.

In an earlier issue of Blood, Chen et al ex-
amined the role of MYH9 in thrombocytopoi-
esis, and showed how myosin–IIA complexes and their upstream signaling pathways regulate megakaryocyte migration and the timing of proplatelet formation. They concluded that a lifting of the restraints imposed on proplate-
let formation by the Rho–ROCK–MLC–myosin–IIA pathway was a key trigger for pro-
platelet formation at the sinusoids. A recent study on mice showed that targeted deletion of myosin–IIA in platelets led to a major prol-
gression of bleeding time and a severe defect in thrombus growth. The lack of myosin–IIA led...
to the absence of shape change and clot retraction in response to platelet agonists, and to platelet morphological changes resembling the human disease. Although MYH9-related human disorders such as May–Hegglin anomaly are autosomal-dominant in inheritance, and therefore myosin-IIA deficiency is less extensive, altered contractile activity during primary hemostasis may contribute to the bleeding episodes that affect some patients.

The current work by Ono and colleagues in Shaun Jackson’s group implies that myosin–IIA complexes play a key role not only in the birth of the platelet, but also in the endgame of hemostatic plug formation and thrombus consolidation in the vessel wall. Because the thrombus is attached to the vessel wall, the contraction will pull the thrombus toward the lesion, simultaneously cementing the breach and facilitating blood flow through the vessel. In the context of atherothrombotic disease, the extent of the contraction may affect the resistance of platelet–rich clots to fibrinolytic agents and to antiplatelet drugs.

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Steps leading to thrombus formation, highlighting the newly described contractile activity that results in a rapidly formed, tightly packed platelet mass independently of fibrin formation.
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