genes.[8] They found that at early time points, significant changes in DNA methylation were achieved. The acquisition of samples at a very early time point was important because if they had waited too long, any differences seen might reflect an alteration in marrow composition (eg, reduced numbers of neoplastic cells). The numbers of patients in their series precluded an assessment of genes that might predict eventual hematologic response or clinical benefit, but their results do suggest the potential of this approach to identify predictors of eventual response or resistance in larger series. Also, data on the mRNA expression changes after treatment were not explored in their report, which is also crucial for understanding mechanisms of response/resistance.

It is clear that MDS is a distinct disease from de novo AML, but there is a good deal of heterogeneity in both categories of disease. Platforms and approaches such as those used by Figueroa et al enable one to peer through an informative looking glass to better clarify epigenetic differences between these 2 diseases based on DNA methylation changes. The same approach can clearly be applied serially in patient populations at risk of evolution to AML, studies that may reveal key epigenetic factors underlying clonal evolution of MDS to AML. When combined with analyses of gene expression and functional assessments of chromatin in normal bone marrow cells, MDS cells, and de novo leukemia cells, these methods should clarify whether there are critical epigenetic events that transform normal hematopoietic cells, induce genetic instability and clonal evolution, enhance survival of new clones, and induce resistance to therapy.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● PLATELETS & THROMBOPOIESIS

Comment on May et al, page 3464

CLEC … too!

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In this issue of *Blood*, May and colleagues[1] demonstrate that the recently described platelet receptor CLEC-2 is important for stabilizing platelet cohesion and thrombus development under flow conditions. The absence of CLEC-2 in vivo is manifested by a continuous release of individual platelets from the growing thrombus and the embolization of small platelet aggregates, resulting in impaired occlusion of damaged vessels in CLEC-2–deficient mice.

The mechanisms that support platelet activation and adhesion to the extracellular matrix have been studied intensively, leading to the identification of a handful of now well-known receptors that participate in each phase of platelet function (reviewed in Kunicki and Nugent[2]). In early stages of platelet recruitment to areas of blood vessel damage, the glycoprotein (GP) Ib complex binds to von Willebrand factor (VWF) and mediates transient platelet attachment to collagens in the extracellular matrix. Platelet GPVI and the integrin $\alpha 2\beta 1$ then become involved to mediate a more stable attachment to collagens and contribute to platelet activation, which is enhanced by the binding of key platelet-activating agonists. Some of the most important agonists, like adenosine diphosphate (ADP) and thromboxane A2, are released from platelets and bind to their cognate receptors. Other platelet agonists, such as thrombin, are produced by the concurrent process of prothrombin conversion, which is accelerated on the activated platelet surface. In the subsequent stages of thrombus formation, activated integrin $\alpha IIb\beta 3$ binds to fibrinogen and/or VWF and mediates platelet cohesion or aggregate formation. We have grown comfortable with these well-described agonists and receptors, but new evidence indicates that our picture of platelet thrombus formation is not quite complete and that additional receptors contribute to this important process.

One of the newest and most exciting developments is the discovery of the contributions of platelet CLEC-2. This membrane receptor was originally identified in immune cells, where its precise function remains unclear.[3] Suzuki-Inoue and colleagues[4] were the first to show that it is also expressed on platelets and represents the receptor bound by the platelet-activating protein rhodocytin, isolated from the venom of the Malayan pit viper *Calloselasma rhodostoma*. Additional studies have demonstrated that podoplanin, expressed by certain tumors, is also a ligand for CLEC-2. Ligand engagement by CLEC-2 causes phosphorylation of a tyrosine residue in the CLEC-2 cytoplasmic domain and subsequent signaling via Syk.[4,5] The identification of the natural ligands that engage and activate CLEC-2 during thrombus formation remains to be determined.

CLEC-2 is a potential novel target for antithrombotic therapy, and evidence in May et al[1] shows that it can be specifically targeted and functionally inactivated in vivo by antibodies, such as INU1. Treatment of mice in vivo with an antibody against CLEC-2 induced a specific and prolonged CLEC-2 deficiency, which was associated with significant protection from occlusive thrombus formation. At the same time, a moderate but significant increase in bleeding times was found in roughly one-half of the

treated mice. In the near future, rational drug design approaches can exploit this knowledge to develop specific inhibitors of CLEC-2 function.

Clearly, there is a new platelet receptor in the picture, reinforcing again the concept that redundancy in platelet-related hemostatic pathways serves to ensure adequate hemostasis while regulating undesirable thrombosis.

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