DNA methylation is a common mode of gene silencing in cancer, and MDS remains the poster child for a disease in which targeting the DNMT enzymes leads to clinical benefit. However, some important questions remain unanswered, two of which are directly addressed in this issue of Blood by Figueroa and colleagues:

What pivotal gene sets are silenced by DNA methylation in MDS and de novo AML, a question that begs a reference to the patterns of normal marrow cells on a genome-wide scale, and what global changes in DNA methylation can be achieved when treating patients with DNMT inhibitors?

Genome-wide microarray technologies have been widely used for well over a decade and allow one to comprehensively assess gene expression. More recently, they have been applied to the study of DNA methylation. There are several ways to assess for DNA methylation changes using microarray-based platforms (see figure). The first uses hybridization of DNA that has been treated with sodium bisulfite, which converts unmethylated cytosines to uracils while methylated cytosines remain cytosines. A second approach uses antibodies directed against 5-methyl-cytosines or methyl-binding proteins to immunoprecipitate methylated DNA fragments. A third uses treatment of the DNA with methyltion-specific restriction enzymes followed by hybridization to microarrays.

In their report, Figueroa et al used an example of the latter technique called HELP (HpaII tiny fragment enrichment by ligation mediated PCR) to clarify the DNA methylation changes that exist in bone marrow samples ranging from normal to myelodysplasia (MDS) to de novo leukemia.

The authors first focused on determining the DNA methylation differences between CD34- and CD34+ cells from bone marrow samples from both normal volunteers and those with MDS. Surprisingly, they found that there was a very high correlation between methylation patterns in CD34- and CD34+ cells within each group. This allowed the authors to examine DNA methylation changes in additional CD34- MDS clinical samples with greater confidence that any differences in methylation between these cells and those from normal or de novo acute myeloid leukemia (AML) samples would not be solely explained by lineage differences. They found that indeed there is a continuum of DNA methylation with the lowest amount of DNA methylation present in normal bone marrow cells, de novo AML cells in the middle, and MDS cells at the methylated end of the spectrum. Although it is unclear whether the DNA hypermethylation seen in MDS may account for the responsiveness of this disease to DNA methyltransferase (DNMT) inhibitors, it is intriguing to speculate that these changes may play a role.

To determine what changes are achievable with epigenetic therapy in humans, the authors next examined bone marrow biopsy specimens taken either before or after treatment with 5-azacytidine in combination with the histone deacetylase inhibitor entinostat; previously, the combination of these two classes of agents was shown to result in synergy of reexpression of DNA hypermethylated...
genes. 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.

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REFERENCES

CLEAR... too!

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In this issue of Blood, May and colleagues1 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 manifest 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 Nugeent). 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 α2β1 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 αIIbβ3 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.1 Suzuki-Inoue and colleagues4 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.1,5 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 al1 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
Through the looking glass

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