which 2 transcriptionally active—and repressive—histone marks are physically linked together. These plastic epigenetic marks prevent undifferentiated ESC differentiation, but in response to environmental cues, they may turn on the specialized transcriptionists to regulate development of more differentiated cells. Although similar epigenetic mechanisms involving “bivalent domains” can occur in adult stem cells, the results of genome-wide approaches to response to environmental cues, they may turn on adult stem cells, the results of genome-wide approaches to better characterize such domains in undifferentiated HSCs are still missing.

In this issue of Blood, Chung et al use a genome-wide comparison of DNA methylation to study epigenetic marks in human umbilical cord (UCB) CD34+ (undifferentiated) and CD34− (differentiated) cells. They found in CD34+ cells a presence of characteristic hypomethylation dip around transcription start sites (TSS) of promoters and hypermethylation in flanking regions. The undermethylated DNA methylation pattern near TSS is different in CpG islands (CGI+) and non-CGI (CGI−) genes and seems to be related to an active gene transcription and dynamic chromatin status in a population of primitive HSCs. Furthermore, undifferentiated HSCs exhibited dynamic open-type chromatin associated with transcriptionally active acetylated histones more than terminally differentiated ones. In the next step, the authors inhibited chromatin condensation (heterochromatin) by preventing methylation of DNA and histones by employing 5-azacytidine or trichostatin A and noticed enhanced self-renewal of murine bone marrow (BM)–derived HSCs that were transplanted into lethally irradiated recipients but not of “steady-state” BM-isolated HSCs. Interestingly, similar treatment of more mature cells leads to partial phenotypic de-differentiation and apoptosis that correlated with the level of their hematopoietic maturation. The authors conclude that the undifferentiated state of hematopoietic cells is characterized by unique epigenetic signature that includes both (1) dynamic chromatin structure and (2) an epigenetic plasticity of gene expression that correlates with the level of differentiation.

This paper is important because it addresses several issues related to developmental biology and potential “plasticity” of HSCs. First, the authors clearly demonstrate the dynamic structure of chromatin and its epigenetic modification during differentiation of hematopoietic cells. Second, data presented explain the efficacy of 5-aza-cytidine and trichostatin A for ex vivo expansion of undifferentiated HSCs at a molecular level. This may be relevant during generation of inducible pluripotent stem cells using the so-called small molecular chromatin modifying agents (eg, BIX-01294 and BayK8644). However, because aberrant epigenetic regulation can lead to tumorigenesis and premature stem cell aging, full understanding of epigenetic processes that are a kind of “2-edged sword” mechanism is a timely challenge. Finally, additional studies on purified HSCs to elucidate biological significance of unique DNA methylation patterns found in current genome-wide analysis will help to better understand epigenetic mechanisms that govern hematopoiesis.

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REFERENCES

CLINICAL TRIALS

Comment on Müller et al, page 4944

Fine-tuning targeted therapy of CML

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In this issue of Blood, Müller and colleagues correlate clinical outcome on dasatinib with baseline BCR-ABL kinase domain mutation status, providing a framework for incorporation of BCR-ABL genotype in choosing the optimal second-line kinase inhibitor therapy in imatinib-resistant patients. This study should help improve outcomes by personalizing therapy based on the mutations detected.

I matinib, the first approved BCR-ABL–selective tyrosine kinase inhibitor, was rapidly established as the preferred front-line therapy for newly diagnosed chronic phase chronic myeloid leukemia (CP-CML) on the basis of its ability to achieve a complete cytogenetic response (CCyR; defined as no detectable Philadelphia chromosome in at least 20 evaluable bone marrow metaphases) in the majority of cases. Unfortunately, it is estimated that imatinib fails to achieve CCyR within 18 months in approximately 25% of patients. These persons are at higher risk for the development of progressive disease, which has been most commonly associated with the development of drug-resistant BCR-ABL kinase domain mutations. Once the clinical importance of kinase domain mutations in BCR-ABL was recognized, 2 second-generation BCR-ABL kinase inhibitors (dasatinib and nilotinib) were clinically developed as they are active against nearly all imatinib-resistant mutations in vitro, with the notable exception of the T315I mutation, which is highly resistant to all 3 approved drugs in vitro.

On the basis of preclinical studies demonstrating the T315I mutation to be the only substitution capable of conferring near-absolute resistance, it was hoped that this mutation would represent the principal clinical weakness of both dasatinib and nilotinib. However, there are clear limits to the predictive power of in vitro models. A true understanding of clinical resistance can be gleaned only from translational studies that interrogate appropriate clinical samples. Encouragingly, several studies have previously found the T315I mutation to represent the primary cause of loss of response to dasatinib, but other mutations at amino acid positions 299 (V299L) and 317 (F317L, F317V) can also be newly detected at the time of disease progression on dasatinib. Gratifyingly, initial preclinical studies demonstrated that of 15 tested mutations, F317L was second only to T315I in the degree of resistance conferred to dasatinib.
To properly integrate preclinical observations into clinical practice, correlative studies of large patient cohorts are essential. To that end, Müller et al evaluated more than 800 patients enrolled in clinical studies with dasatinib following imatinib failure, and in 384 patients with detectable mutations at baseline, 63 different mutations were identified. Of mutations detected in at least 5 patients, 3 were associated with a particularly low probability of likelihood of CCyR achievement on dasatinib: T315I (0/21 cases), F317L (1/14 cases), and Q252H (1/6 cases). Similarly, a recent publication of imatinib-resistant CP-CML patients treated with nilotinib has revealed a handful of problematic mutations in addition to T315I (Y253H, E255K, E255V, F359C, F359V). Again, it is scientifically satisfying that these mutations are the most relatively resistant to nilotinib after the T315I mutation. Although neither report demonstrated a significant difference in outcome based on the presence or absence of any mutation at baseline, it is becoming clear that patients who harbor specific mutations may, in fact, be better served by treatment with a particular second-line agent.

A notable finding of the Müller et al study is the relative incidence of mutations that are potentially problematic to second-line therapies. Of the 384 patients with mutations, 42 patients had 1 of the 4 mutations documented to respond poorly to dasatinib, while 103 had mutations that do not respond well to nilotinib, a finding that is in general agreement with other studies that have assessed the relative frequencies of imatinib-resistant mutations in large cohorts of patients. This higher degree of cross-resistance between imatinib and nilotinib (relative to dasatinib) is not surprising given the degree of structural similarity between imatinib and nilotinib. However, it must be noted that no randomized controlled studies comparing dasatinib and nilotinib in patients with imatinib failure have been performed, and both agents achieve CCyR in a substantial proportion of CP-CML patients with imatinib resistance.

Nonetheless, the findings of Müller et al and Hughes et al provide clinicians with guidance regarding the preferential choice of a second-line agent. For imatinib-intolerant patients, there is no clinical evidence at this time to support either dasatinib or nilotinib, and weighing individual comorbidities with drug side effect profiles can be important. It is interesting that both Müller et al and Hughes et al identified BCR-ABL mutations in a small but significant proportion of imatinib-intolerant patients (8% and 10%, respectively), suggesting that some patients with imatinib-“intolerant” disease have evolved subclinical imatinib resistance, and screening imatinib-intolerant patients, in addition to imatinib-resistant patients, for BCR-ABL kinase domain mutations may further guide clinicians in pursuit of the optimal second-line therapy.

Because CML represented the very first human malignancy to be treated with small-molecule tyrosine kinase inhibitors, it is hoped that the clinical experience with imatinib, dasatinib, and nilotinib will establish a paradigm that can be applied to this burgeoning class of molecules. With the recent work of Müller et al and Hughes et al, the era of personalized medicine for CML has arrived.

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REFERENCES

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