To the editor:

DAP-kinase CpG island methylation in acute myeloid leukemia: methodology versus biology?

Acute myeloid leukemia (AML) is a clonal disorder evolving from myeloid progenitor cells on the background of a number of genetic changes including balanced translocations1,2 and alterations of myeloid progenitor cells on the background of a number of genetic changes.

References


Note: Prior to receiving Teitelbaum et al’s letter and Bergsagel et al’s response, the Editorial Board of Blood initiated a new policy requiring disclosure of any significant financial interests in subjects covered in the text of published papers in the Journal. This policy is stated in the Author Guide of this and other issues of Blood. However, this disclosure policy was not in place at the time of writing of the review “Benzene and multiple myeloma,” which has prompted the above exchange of letters.—Editor
Response:

DAP-kinase methylation: methodology and biology

The above letter by Aggerholm et al examines the methylation of DAP-kinase in acute myelogenous leukemia (AML). As they have done for the tumor suppressor gene p15/CDKN2B, they compare 2 methods of determining methylation patterns: Methylation specific PCR and DGGE, a method they have developed. The more sensitive method (MSP) yields a higher prevalence of methylation at this loci, and the authors rightly question the importance of this methylation. Why do these results differ?

In our original report, we focused primarily on acute lymphoblastic leukemia and lymphomas, as our initial studies suggested that methylation was particularly frequent in these malignancies. Furthermore, we observed that this methylation was limited to transformed cells, and was not observed in normal peripheral leukocytes or in EBV immortalized B lymphocytes, even with the sensitivity of MSP as carried out in our lab. This specificity for the transformed phenotype, the association of the lack of expression with inhibited γ-interferon induced programmed cell death, and the further specificity for B vs T-cell malignancies all suggested an important role for this gene in these malignancies.

We did examine other malignancies, including acute myelogenous leukemia, as studied by Aggerholm. In the 26 pediatric AML samples examined, only one exhibited DAP-kinase methylation, and though this leukemia was more correctly classified as a biphenotypic leukemia, as studied by Aggerholm. In the 26 pediatric AML samples, the vast majority of amplified DAP-kinase alleles migrated to a position in the gel corresponding to unmethylated DNA (Figure), and a band pattern consistent with some degree of DAP-kinase promoter methylation could be demonstrated in only 1 case of adult AML (data not shown). For comparison, the 10 AML samples represented in the Figure showed extensive methylation of the p15/CDKN2B CpG island by bisulfite-DGGE analysis (Figure).

The direct comparison between the MSP and DGGE assays performed in this study prompts us to suggest that silencing of DAP-kinase by methylation may not be a biologically significant event in AML. This is evidenced by the fact that by MSP analysis, 42% of adult AML cases showed indication of DAP-kinase CpG island methylation, whereas bisulfite-DGGE of the same samples revealed that only an insignificant fraction of leukemic blasts, or possibly even a different cell type, contained methylated DAP-kinase alleles. These contrasting results may be reconciled by data in a recent study where we found that a small fraction of normal lymphocytes are methylated in the promoter region of p15/CDKN2B. The presence of rare nonmalignant methylated cells in clinical leukemia samples implies that highly sensitive methods for detection of methylation, such as the MSP, should be employed with caution and preferably in combination with other methods in order to obtain information on both the fraction and the extent of methylated alleles. In this setting, and when seen in conjunction with accepted clinical correlates, promoter methylation could, however, well turn out to be of significance in stratifying patients with hematologic diseases.

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References