10. The question of whether rituximab can improve results of CHOP-14 will not be answered until the analysis of the completed RICOVER-60 trial (1220 patients), which should be available in summer 2005. Moreover, there are no data on the addition of rituximab to intensified chemotherapy regimens in young high-risk patients, and the respective Mega-CHOEP (CHOP + etoposide) trial of the DSHNHL has just started recently.

11. Before the results of the RICOVER-60 and Mega-CHOEP trials are available, there is no scientifically justified claim of a “standard care” for elderly patients and young high-risk patients with DLBCL.

In summary, treatment of aggressive lymphoma is more complex than Coiffrer and Salles try to make us believe. For the time being, the conclusion of Coiffrer’s and Salles’ letter with the generalizing claim of a “standard care in DLBCL” lacks scientific evidence and must be rejected.

Michael Pfreundschuh and Markus Loeffler, for the German High-Grade Non-Hodgkin’s Lymphoma Study Group (DSHNHL)

References


To the editor:

Low level of DAP-kinase DNA methylation in myelodysplastic syndrome

Recently, Voso et al reported the frequent hypermethylation of the DAP-kinase gene in myelodysplastic syndrome (in 16 of 34 samples).

Since we are interested in the epigenetic profile of myelodysplastic syndrome (MDS), we assessed the methylation status of the DAP-kinase gene in a larger series of 73 bone marrow biopsies (26 refractory anemia [RA]; 26 RA with ringed sideroblasts [RARS], and 21 RA with blast excess [RAEB]), using exactly the same DAP-kinase tic syndrome (MDS), we assessed the methylation status of the gene in myelodysplastic syndrome (in 16 of 34 samples). In 43% of all MDS biopsies analyzed, a signal for methylated DNA could be detected. This frequency is very similar to the one reported by Voso et al.

Using real-time quantitative reverse transcription–PCR, we also measured the DAP-kinase mRNA expression level in 18 of 20 control biopsies, 12 MDS samples without any methylation, and 26 of 31 MDS samples displaying methylation signals. No significant reduction in DAP-kinase mRNA level could be observed. On the contrary, we found a weak trend toward increased DAP-kinase mRNA expression in the MDS biopsies in comparison with the control group (Figure 1B), which fits well to the proapoptotic function of death-associated protein kinase (DAP)–kinase and the well-known increase in apoptosis in the bone marrow of MDS patients. Parker et all clearly showed in their study, which is cited by Voso et al as reference 21, that the rate of apoptosis is significantly increased in CD34+ cells in RA, RARS, and RAEB. Therefore, this study rather contradicts than supports the statement that “a common feature of MDSs is a decreased apoptosis rate in bone marrow progenitor cells.”

Since Voso et al provide no details of the reaction conditions and do not show any primary data, evaluation of the reported results is difficult. From the context of paragraph one in “Results and discussion,” we assume that Voso et al carried out their mRNA expression studies with a subset of acute myeloid leukemia (AML) samples and not with MDS samples, which could explain in part the discrepancy of the results.

The well-known differences in the apoptosis rate of RA, RARS, and RAEB versus RAEB in transformation (RAEB-t), secondary AML, and AML might explain differences in the epigenetic inactivation pattern of the DAP-kinase gene in these entities, which has to be addressed using quantitative methylation and expression

Figure 1. Methylation and expression analysis of DAP-kinase in MDS. (A) Results of quantitative methylation analysis of the DAP-kinase gene in MDS patients and control cases. (B) Expression analysis of DAP-kinase gene in MDS. Measurement of DAP-kinase mRNA levels using quantitative real-time PCR methodology. The mean expression level of the control group was set to 100% and all individual expression levels were calculated to this mean using the ΔΔCt–method. The mean relative expression level of the MDS samples is 127% (P = .5, Mann-Whitney test). Transcript levels were normalized to j1-glucuronidase (j1-GUS).
assays. Long-term follow-up studies will be necessary to evaluate the significance of low-level methylation in MDS for the clonal evolution to AML.

Finally, we would like to mention that 4 years ago in Blood, Aggerholm et al. raised the question of overestimating the proportion of DAP-kinase gene methylation in AML by using methylation-specific PCR.

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References


Response:

DAP-kinase hypermethylation in MDS

In their letter, Brakensiek et al comment on our recently published paper on DAP-kinase hypermethylation in therapy-related acute myeloid leukemia (AML) and myelodysplastic syndromes (MDSs). They report that DAP-kinase was hypermethylated in a proportion of MDS similar to the one we reported (43% vs 47%). Using a real-time quantitative approach, they extend this observation, showing that the frequency of methylation is less than 5% and question the biologic significance of DAP-kinase hypermethylation in MDS. We think that this is an interesting finding, and, since the bone marrow cell distribution in MDS is heterogeneous, studies are warranted to evaluate the role of DAP-kinase methylation in this disease, by analyzing bone marrow subpopulations, including CD34+ cells, their progeny, and CD19+ B lymphocytes.

Low levels of DAP-kinase hypermethylation (0.003%-1.181%) have been reported in normal lymphocytes, especially in selected B cells (1%-6%), using a real-time methylation-specific polymerase chain reaction (MSP). We did not find any DAP-kinase hypermethylation in mononuclear cells isolated from 13 bone marrow and 15 peripheral blood samples from healthy individuals, of age similar to that of the patients, making it unlikely that the methylation we see in MDS could be due to contaminating B lymphocytes. Furthermore, the sensitivity of our polymerase chain reaction (PCR) technique might be too low to detect methylation at the low levels described by Reddy et al. We performed a dilution curve on 2 independent samples, in which we diluted a completely methylated sample into an unmethylated DNA: a distinct band was visible up to a dilution of 1:16 (6.75% positive; Figure 1A).

Apoptosis is truly an issue in MDS. Parker et al. show that in MDS early disease is associated with excessive apoptosis and elevated ratio of apoptosis to proliferation. Increased proliferative rates are observed in RAEB, whereas leukemic transformation arises through inhibition of apoptosis. We agree that it would have been more correct to say “a common feature of high-risk MDSs is a decreased apoptosis rate in bone marrow progenitor cells.”

We used the methylation-specific PCR conditions reported by Katzenellenbogen et al., and data were not shown due to the limited space available for “Brief reports.” Figure 1B shows an example of MSP for unmethylated and methylated DAP-kinase.

Since bone marrow of patients with MDS is heterogeneous for cell content, we used for our expression analysis mRNA extracted from 37 bone marrow mononuclear cells, which usually contained more than 50% blasts, of patients with AML at the time of initial diagnosis. We showed that hypermethylation correlated to loss of expression. Aggerholm et al.1 raised the question that MSP may overestimate the proportion of DAP-kinase gene methylation in AML, as they found methylation in 19 of 45 AML cases using MSP—confirming this finding by sequencing the PCR products—but only in 1 of 49 cases using a different technique (bisulfite denaturing gradient gel electrophoresis [DGGE]). We (and many other authors) could confirm the functional role of DAP-kinase methylation, studied by MSP, by the corresponding lack of expression by reverse transcription (RT)–PCR. We agree that longitudinal studies from early disease to leukemic transformation, also using quantitative methylation assays, will help to clarify the

References

contribution of DAP-kinase methylation to the pathogenesis and evolution of MDS and AML.

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References


To the editor:

Prenatal origin of GATA1 mutations may be an initiating step in the development of megakaryocytic leukemia in Down syndrome

Recently, somatic mutations in exon 2 of the transcription factor GATA1 gene have been detected in essentially all Down syndrome (DS) megakaryocytic leukemia (AMkL) and transient myeloproliferative disorder (TMD) cases. This is the most specific genetic abnormality other than trisomy 21 in DS AMkL cases and is likely linked to the estimated 500-fold higher risk of DS children to develop AMkL compared with non-DS children. In this study, GATA1 mutations were screened in hematopoietic tissues from DS fetuses and infants that had no pathologic evidence of leukemia to establish the stage of development at which GATA1 mutations may arise.

DS liver and/or bone marrow samples were obtained from archival autopsy specimens (ages, 10 days to 10 months) from the Department of Pediatric Pathology, Children’s Hospital of Michigan and fetal liver tissue blocks, from therapeutically terminated pregnancies (gestational ages, 18-23 weeks) from the Department of Pathology, Hutzel Women’s Hospital. The research protocol was approved by the institutional review boards of Wayne State University and the University of Chicago. Following deparaffinization according to the manufacturer’s instructions (Qiagen, Valencia, CA), genomic DNA was isolated by standard techniques. All fetuses and infants were confirmed to have trisomy 21 by standard karyotype analysis. Screening for GATA1 exon 2 mutations was performed by single-strand polymorphism assay (SSCP) as previously described. Altered migration products were excised, and DNA was eluted and amplified by PCR and then sequenced.

GATA1 mutations were detected in 2 of 9 liver samples from fetuses (gestational ages, 21 and 23 weeks) and in 2 of 5 DS infant autopsy bone marrow samples (ages, 4 and 6 months; Table 1). It is unknown whether the fetuses would have survived to term without the development of TMD and/or AMkL, though the detection of GATA1 mutations in hematopoietic tissues obtained postnatally suggests that mutations may exist in the absence of leukemia and are likely early leukemogenic events in DS. Although no mutations were detected in 60 peripheral blood samples of healthy DS children, this does not exclude the possibility that mutations were present though below the sensitivity of our assay or detectable only in bone marrow hematopoietic cells.

The uniform detection of GATA1 mutations in DS children with TMD and AMkL suggests that trisomy 21 may be associated with an increased mutation rate in DS as reported by Finette et al. Increased expression of the cystathionine-β-synthase gene (localized to 21q22.3) in the fetal liver and the known origin of TMD in the fetal liver may result in an increased mutation rate due to a “functional folate deficiency” state. Mutations may arise in other unidentified genes in DS tissues, though GATA1 mutations may confer a proliferative advantage allowing for the expansion and survival of GATA1 mutant-containing clones. A case of identical twin DS infants with TMD with the same GATA1 mutations suggests that the GATA1 mutation arose in utero. Furthermore, GATA1 mutations have been detected at birth in Guthrie newborn screening cards from DS infants who later developed AMkL. Since peripheral blood smears were not examined in this latter study, one cannot rule out the possibility that the infants had TMD.

Although the sample size of our study is small, the frequency of GATA1 mutations detected in the fetal and neonatal liver/bone marrow samples appears higher than the incidence of TMD or AMkL in DS children. These results demonstrate that the acquisition of GATA1 mutations can occur prenatally, mutations can exist in the absence of leukemia and are likely early steps in a multistep process of leukemogenesis, and additional genetic events and/or environmental exposures are likely necessary for the full development of leukemia in DS.

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Table 1. Summary of GATA1 mutations detected in down syndrome fetal and infant hematopoietic tissues

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age</th>
<th>Mutation</th>
<th>Last normal amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal liver 21</td>
<td>21 wk*</td>
<td>240insG; 273del 2 bp</td>
<td>Asp42</td>
</tr>
<tr>
<td>Fetal liver 23</td>
<td>23 wk*</td>
<td>318del 13bp</td>
<td>Ala68</td>
</tr>
<tr>
<td>Infant bone marrow 4 mo</td>
<td>4 mo</td>
<td>299ins 4 bp</td>
<td>Tyr62</td>
</tr>
<tr>
<td>Infant bone marrow 6 mo</td>
<td>6 mo</td>
<td>299insT</td>
<td>Tyr62</td>
</tr>
</tbody>
</table>

*Gestational age.
Low level of DAP-kinase DNA methylation in myelodysplastic syndrome

Kai Brakensiek, Florian Länger, Hans Kreipe and Ulrich Lehmann