We have shown that the abnormalities of 12p are relatively common in adverse risk AML. These abnormalities might have some effects on disease progression. Finally, since SKK-1 cells probably contain undetected genetic alterations which could influence the progression of MDS to AML, analyzing this cell line may reveal alterations and their role in leukemogenesis.

Hiroshi Matsuoka,* Tokuhide Murayama,* Tami Kaizumi,* Ryuichiro Nishimura,* Ryoji Kawauchi,* Toshitario Nakagawa*

*Hematology/Oncology Division, Department of Medicine, Hyogo Medical Center for Adults, Akashi, ‡Hyogo Institute of Clinical Research, †Center for Molecular Biology and Cytogenetics, SRL, Tokyo, Japan

Acknowledgments: we thank the medical, nursing and laboratory staff of the Hyogo Medical Center for Adults for their excellent cooperation. We are also grateful to Mr. Yoshikazu Mimura and Mrs. Kumi Numata for photographic work and technical assistance.

Key words: trisomy 8, myeloid cell line, SKK-1, leukemogenesis AML, MDS.

Correspondence: Tokuhide Murayama, Hematology/Oncology Division, Department of Medicine, Hyogo Medical Center for Adults, 43-70, Kita-Oji, Akashi, Hyogo, 673-8558, Japan.

Phone: international +81.78.9294154. Fax: international +81.78.9292387. E-mail: tmurayam@hp.pref.hyogo.jp

References


CYP1A1 belongs to the cytochrome P450 family and is a phase I detoxification enzyme involved in the bioactivation of several chemical carcinogens, including cytotoxic drugs. Cytochrome P450 enzymes transfer electrons onto toxicants to create highly reactive intermediates which are usually coupled to glutathione or other groups, producing water-soluble compounds, but can also interact with DNA, resulting in the formation of DNA adducts.

Polymorphisms in carcinogen- and drug-metabolizing enzymes may increase the risk of acute myeloid leukemia (AML) and may influence prognosis. We report that the polymorphic variant of the cytochrome P450 CYP1A1*2A, present in 11.3% of patients, is an independent unfavorable prognostic factor for failure-free and overall survival in patients with AML.

CYP1A1*2A variant allele identified a prognostically unfavorable group of patients, with a shorter failure-free survival (FFS) and overall survival (OS) (Figure 1), than patients with the wild type. The CYP1A1*2B and *4 alleles did not have any prognostic relevance. Age over 60 years and WBC > 30×10⁹/L were negative prognostic factors (FFS and OS: \( p = 0.0005 \) and \( p = 0.01 \) for age and \( p = 0.07 \) and \( p = 0.04 \), for WBC, respectively). Karyotype was available for 81 patients and had a negative prognostic value, with a significant difference in FFS and OS for patients with favorable (n=26), intermediate (n=43) and adverse (n=12) karyotype (\( p = 0.0001 \) and \( p = 0.0002 \), as defined by Grimwade et al.\(^8\)).

FLT3-ITD, analyzed by PCR,\(^7\) were present in 16 (16.5%) patients and these patients had a significantly shorter FFS and OS than did patients without FLT3-ITD (\( p = 0.003 \) for both).

Multivariate analysis using the Cox regression model and including the CYP1A1*2A allele, FLT3-ITD, age, WBC and cytogenetic risk groups, showed that CYP1A1*2A and age were independent prognostic factors for survival (Table 1). Probably due to the limited number of patients analyzed, karyotype, FLT3-ITD and WBC were not prognostic factors. To our knowledge, this is the first study assessing the prognostic value of cytochrome P450 polymorphisms in AML. Since the CYP1A1 variants *2A and *2B have increased activity and/or inducibility, the increased production of electrophilic agents may contribute to the accumulation of genetic changes. Bowen et al.\(^7\) showed that the CYP1A1*2B variant is common in a group of AML characterized by RAS mutations and complex karyotype, but not in those with FLT3-ITD.\(^6\) Data on CYP1A1*2A were not reported.\(^6\) We did not find any associations between FLT3-ITD and CYP1A1 detoxification enzymes polymorphisms (data not shown). Similarly, GST polymorphisms (GSTM1/GSTT1 deletions and the GSTP1 Ile105Val mutation), previously determined for this cohort of patients,\(^3\) were also not associated with FLT3-ITD (data not shown).

Genotyping of detoxification polymorphisms might complement diagnostic cytogenetics and FLT3 mutation analysis, and may ultimately permit an individualized treatment approach in patients with AML.

Maria Teresa Voso, Francesco D’Alò, Daniela Gumiero, Francesco Guidi, Stefan Hohaus, Giuseppe Leone

Istituto di Ematologia, Università Cattolica S. Cuore, Rome, Italy

Funding: this work was supported by grants from M.U.R.S.T (Ministero dell’Università e della Ricerca Scientifica e Tecnologica) and A.I.R.C. (Associazione Italiana per la Ricerca sul Cancro).

Key words: acute myeloid leukemia, CYP1A1 polymorphisms, P450, prognosis.

Correspondence: Dr. Maria Teresa Voso, Istituto di Ematologia, Università Cattolica S. Cuore, largo A. Gemelli 4, 00168 Rome, Italy. Phone: international +39.06.30154180. Fax: international +39.06.35503777. E-mail: mtvos@rm.unicatt.it

References

1. Ingelman-Sundberg M. Genetic susceptibility to adverse effects of drugs and environmental toxicants. The role of the CYP family of enzymes. Mutat Res 2001;482:11-9.

Table 1. Multivariate analysis of factors predicting survival.

<table>
<thead>
<tr>
<th></th>
<th>Failure-free Survival</th>
<th>Overall Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio (95% C.I.)</td>
<td>Hazard Ratio (95% C.I.)</td>
</tr>
<tr>
<td>CYP1A1*2A</td>
<td>0.013</td>
<td>2.7</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>0.47</td>
<td>0.3</td>
</tr>
<tr>
<td>Cytogenetic risk group</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Age</td>
<td>0.001</td>
<td>3.1</td>
</tr>
<tr>
<td>WBC count</td>
<td>0.06</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Figures

Figure 1. The CYP1A1*2A allele predicts for poor outcome. Outcome of patients who were carriers of the CYP1A1*2A allele (n=11) was compared to that of patients who did not present this polymorphism (n=86). Survival curves were estimated using the Kaplan-Meier product limit method. Differences in the survival curves were evaluated using the log-rank test. A. Failure-free survival. B. Overall survival.

Table 1. Multivariate analysis of factors predicting survival.
Acute Myeloid Leukemia

Multiplex reverse transcription polymerase chain reaction screening in acute myeloid leukemia detects cytogenetically unrevealed abnormalities of prognostic significance

A commercial multiplex reverse transcription polymerase chain reaction screening assay, covering 28 leukemic fusion transcripts, was applied in 143 samples obtained from patients with acute myeloid leukemia at primary diagnosis. In five patients, a cytogenetically unrevealed fusion gene of prognostic importance was detected, while the assay failed to detect one case of t(15;17).

Current prognostic stratification of acute myeloid leukemia (AML) at diagnosis is based on conventional cytogenetics. Recognition of patients with the abnormalities t(8;21), t(15;17) and inv(16) is of particular interest in AML prognostication because these abnormalities are associated with relatively favorable prognosis.

Table 1. Fusion transcripts detected in 143 samples by the multiplex RT-PCR screen test.

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Fusion transcript</th>
<th>Adults (N=132)</th>
<th>Children (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;19)(q23;p22)</td>
<td>MLL1/AF9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t(11;19)(q23;p22)</td>
<td>MLL1/AF1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t(11;19)(q23;p22)</td>
<td>E2A/PEX1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>AML1/CAP/MDS1/EVI1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>NPM/MLF1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t(4;11)(q21;p33)</td>
<td>MLL1/AF4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t(15;12)(q33;p13)</td>
<td>TEL/PDGFRB</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t(5;17)(q35;p21)</td>
<td>NPM/RARc</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*The screening test covers the BCR/ABL variants: b2a2, b3a2, c2a2, b2a3, b3a3, e1a2, e6a2, e1a1. **t(9;22)(q34;q11) BCR/ABL el1a2 were detected in one case, t(9;22)(q34;q11) BCR/ABL el2a and t(9;22)(q34;q11) BCR/ABL el2a were detected in one case.

Current prognostic stratification of acute myeloid leukemia (AML) at diagnosis is based on conventional cytogenetics. Recognition of patients with the abnormalities t(8;21), t(15;17) and inv(16) is of particular interest in AML prognostication because these abnormalities are associated with a relatively favorable prognosis. Conventional karyotyping may be hampered by insufficient quality or number of metaphases, low sensitivity or selective outgrowth. Furthermore, translocations of prognostic significance may be cytogenetically undetected if they involve regions with similar band patterns. The aim of this study was to evaluate the additional prognostic information obtained by multiplex reverse transcription polymerase chain reaction (RT-PCR) screening for leukemia-associated genes with a commercially available assay. Multiplex RT-PCR screening for translocations is independent of dividing cells, has a high level of sensitivity, and may identify translocations that are not detected by conventional karyotyping. The HemaVision® Multiplex-RT-PCR Screen Test (DNA Technology, Aarhus, Denmark) detects 28 of the most common leukemic fusion genes and more than 80 splice variants (Table 1). In brief, reverse transcription is performed with a mixture of translocation-specific primers. PCR amplification is performed in two steps: a master PCR amplification followed by nested PCR which screens for the presence of fusion transcripts and a split-out PCR amplification followed by nested PCR which identifies the specific fusion transcript (6). Each of the 8 parallel nested multiplex master PCR reactions contains a mixture of primer pairs for the detection of several fusion transcripts and two primer pairs for an internal control gene product of 911 base pairs. When the presence of one or more fusion transcripts is detected by one or more master PCR reactions, the corresponding split-out reactions with individual primer pairs are performed.

To assess the additional prognostic information obtained by the multiplex RT-PCR screening test, we performed a retrospective study of 143 patients with a median age of 63 years (range 0-85) (132 adults, 11 children). Inclusion criteria were: (i) adults diagnosed with AML at Herlev Hospital, Denmark, during a 12-year period from...