p16 Gene Homozygous Deletions in Acute Lymphoblastic Leukemia

By Bruno Quesnel, Claude Preudhomme, Nathalie Philippe, Mickael Vanrumbeke, Isabelle Dervite, Jean Luc Lal, Francis Bauters, Eric Wattel, and Pierre Fenaux

The p16 protein is a cyclin inhibitor encoded by a gene located in 9p21, which may have antioncogenic properties, and is inactivated by homozgyous p16 gene deletion or, less often, point mutation in several types of solid tumors often associated to cytogenetic evidence of 9p21 deletion. We looked for homozgyous deletion and point mutation of the p16 gene in acute lymphoblastic leukemia (ALL), where 9p21 deletion or rearrangement are also nonrandom cytogenetic findings. Other hematologic malignancies including acute myeloid leukaemia (AML), myelodysplastic syndromes (MDS), chronic lymphocytic leukaemia (CLL), and myeloma were also studied. Homozgyous deletion of the p16 gene was seen in 9 of the 63 (14%) ALL analyzed, including 6/39 precursor B-ALL, 3/12 T-ALL, and 0/12 Burkitt's ALL. Three of the 7 ALL with 9p rearrangement (including 3 of the 5 patients where the 9p rearrangement was clearly associated to 9p21 monosomy) had homozgyous deletion compared to 5 of the 55 patients with normal 9p (the last patient with homozgyous deletion was not successfully karyotyped). Single stranded conformation polymorphism analysis of exons 1 and 2 of the p16 gene was performed in 88 cases of ALL, including the 63 patients analyzed by Southern blot. Twenty-six of the cases had 9p rearrangement, associated to 9p21 monosomy in at least 12 cases. A missense point mutation, at codon 49 (nucleotide 164), was seen in only 1 of the 88 patients. No homozgyous deletion and no point mutation of the p16 gene was seen in AML, MDS, CLL, and myeloma.

Homozygous deletion of interferon α genes (situated close to p16 gene in 9p21) was seen in only 3 of the 9 ALL patients with p16 gene homozgyous deletion, and none of the ALL without p16 gene homozgyous deletion. Our findings suggest that homozgyous deletion of the p16 gene is seen in about 15% of ALL cases, is not restricted to cases with cytogenetically detectable 9p deletion, and could have a pathogenic role in this malignancy. On the other hand, p16 point mutations are very rare in ALL, and we found no p16 homozgyous deletions or mutations in the other hematologic malignancies studied.

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Table 1. Primers Used for the PCR of Exons 1 and 2 of the p16 Gene

<table>
<thead>
<tr>
<th>DNA Fragment Amplified</th>
<th>Fragment Size</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Region 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(containing exon 1)</td>
<td>343 bp</td>
<td>2F 5’GAGAAGAAGGAGGGGCTG 3’</td>
</tr>
<tr>
<td><strong>Region 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(containing exon 2)</td>
<td>394 bp</td>
<td>P36 5’TTCCTTCTCGTACGCGGC 3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M1 5’GTACAAATTCCGACTGTCGCTTTC 3’</td>
</tr>
</tbody>
</table>

According to French-American-British criteria,12,13 and an immunophenotype was performed in all cases. All patients, except one case of ALL, were successfully karyotyped by conventional banding techniques.14

In the 63 ALL patients who underwent Southern blot analysis, DNA was extracted from blood or bone marrow (BM) cell samples containing greater than 80% blasts. SSCP analysis was made in those 63 patients, and in 25 additional cases of ALL that generally had cytogenetic rearrangements involving 9p and where DNA could only be obtained by scraping cells from marrow slides, as previously described.13 This method, in our experience, generally provides sufficient DNA for PCR-SSCP analysis, but not for Southern blot analysis.

DNA for Southern blot analysis was obtained from blood samples in CLL (containing 63% to 90% lymphocytes), marrow samples in MDS (containing 74% to 92% myeloid cells), and myeloma (containing 50% to 63% of plasma cells), and blood or marrow samples in AML (containing at least 70% blasts). In additional cases of AML, MDS, CLL, and myeloma, DNA was obtained for PCR-SSCP analysis by scraping marrow cells, as for ALL.

Southern blot analysis of IFNa genes was also performed in the 63 cases of ALL studied by Southern analysis of the p16 gene. Finally, Southern blot analysis of the p16 gene was made in HSB2 and CEM cell lines, two T-ALL cell lines obtained from American Type Culture Collection (ATCC; references CLL 120.1 and CCL 119, respectively).

**Methods**

**Southern blot analysis.** DNA was digested with EcoRI and HindIII restriction enzymes, separated by electrophoresis in 0.8% agarose gel and transferred to nylon membranes, according to conventional methods.15

The p16 gene probe was used was a 0.96 cDNA probe (kindly provided by D. Beach, Cold Spring Harbor, NY). Southern blots of ALL cases were subsequently rehybridized to an IFNa-1 EcoRI-Xba I 642-bp genomic probe (kindly provided by M. Tovey, CNRS UPR 274, Villejuif, France) which cross-hybridizes with all IFNa genes. Finally, blots were hybridized to a probe for the actin gene, situated on chromosome 7, which served as control. In a few cases of MDS and AML with monosomy 7, a probe for the neurofibromatosis (NF1) gene, which is not deleted in our experience in adult MDS and AML, and which is situated on chromosome 17, was used instead of the actin probe.17

Homozygous deletion of the p16 and IFNa genes was determined by visual inspection of the autoradiographs sequentially hybridized with the p16, IFNa, and control probe. Suspected homozygous deletions were more objectively confirmed by measuring the intensity of hybridization signals by a densitometer (Densyslab; BIOPROBE Systems, Montreuil, France).

**PCR-SSCP analysis.** Intronic oligonucleotide primers were purchased from BIOPROBE Systems. The names and nucleotide sequences of the primers used in this work are listed in Table 1. Two genomic regions were amplified: region 1, encompassing exon 1 and measuring 343 bp; and region 2, encompassing exon 2 and measuring 394 bp. For region 1, we used the primers published by Kamb et al.1 Because SSCP analysis seems to require fragments of no more than 350 to 400 bp in length, region 2 was digested, after amplification and before SSCP analysis, by Smal I enzyme, because an Smal I restriction site is present in exon 2. This led to two fragments, region 2a and region 2b, each measuring 162 and 232 bp, respectively.

Genomic DNAs (0.1 μg) were subjected to PCR in a 50-μL solution containing 200 μmol/L of each of dATP, dGTP, dTTP, dCTP, 0.1 μL of 32P-dCTP (Amersham, Amersham, UK, 10 μCi/μL), 25 pmol of 5’ and 3’ primer 5 dimethyl sulfoxide (DMSO), 10 mmol/L TRIS-HCl (pH 9), 50 mmol/L KCl, 1.5 mmol/L MgCl2, Triton ×100 (0.1%) and gelatin 0.2 mg/L, 0.4 U of Taq polymerase (Appigene, Illkirch, France) in a thermostorer (Mimicycler; M.I. Research, Watertown, MA). For exon 1, PCR was performed as follows: 10 minutes at 94°C, then 20 cycles with 94°C for 1 minute, 72°C for 1 minute with decrement of 0.2°C per cycle followed by 15 cycles with 94°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute, then by final elongation at 72°C for 5 minutes. For exon 2, PCR was performed as follows: 10 minutes at 94°C, then 20 cycles with 94°C for 1 minute, 56°C for 1 minute, 72°C for 1 minute, with a decrement of 0.2°C per cycle followed by 15 cycles with 94°C for 1 minute, 52°C for 1 minute, 72°C for 1 minute, followed by final elongation at 72°C for 5 minutes. After amplification, 1 μL of the reaction mixture for region 1 was mixed with 19 μL of 0.1% sodium dodecyl sulfate (SDS), 20 mmol/L EDTA solution. For region 2, 1 μL of the reaction mixture was first digested by Smal I in 10 μL, and digested in 10 μL of SDS-EDTA solution. Then 3 μL of the digested region 1 and of regions 2a and 2b, respectively, were mixed with 3 μL of a solution of 95% formamide, 20 mmol/L EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol, heated 4 minutes at 80°C and applied (2 μL/ lane) to an MDE polyacrylamide gel containing 7 mol/L urea.

**Sequencing analysis.** PCR amplification was performed as described above, using a biotinylated primer. Single-stranded DNA template was obtained by binding the biotinylated PCR products to streptavidin-coupled magnetic beads (Dynabeads; Dynal, Oslo, Norway) and NaOH denaturation according to the manufacturer. Sequencing reactions were performed following the Sequenase 2.0 protocol (US Biochemical Corp, Cleveland, OH). The sequencing primer was the nonbiotinylated primer used in the PCR reaction. When ambiguities were present, the opposite strand was sequenced using the reciprocal combination of biotinylated and nonbiotinylated primers. The sequencing products were analyzed on a 6% polyacrylamide gel containing 7 mol/L urea.

**RESULTS**

**Southern Blot Analysis of the p16 Gene in ALL.**

The 63 ALL studied by Southern blot included 2 children ages ≤15 years and 61 people ages >15 years, 24 females and 39 males, 39 B-precursor ALL (37 early B-ALL, which were CALLA+ in 31 cases, and 2 pre B-ALL with intracytoplasmic IgS), 12 T-ALL, and 12 Burkitt’s ALL (Table 2). Cytogenetic analysis (successfully performed in 62 cases) was normal in 16 cases. Four patients had a hyperdiploid karyotype, 6 had t(9;22), 4 had t(4;11), 12 had t(8;14), 2 had t(1;19), and the remaining patients had various other rearrangements. Seven patients had 9p rearrangements, including unbalanced translocations between chromosome 9...
Table 2. Hematologic Characteristics of the Patients Studied by Southern Analysis of the p16 Gene

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Patients Studied</th>
<th>No. of Cases With 9p Rearrangement*</th>
<th>No. of Cases With Homozygous p16 Gene Deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL (39 B precursor ALL, 12 T-ALL, 12 Burkitt's ALL)</td>
<td>63</td>
<td>7 (5)</td>
<td>9</td>
</tr>
<tr>
<td>AML (1 M1, 3 M2, 1 M3, 2 M4, 3 M5, 1 M6)</td>
<td>11</td>
<td>2 (2)</td>
<td>0</td>
</tr>
<tr>
<td>MDS (8 RA, 9 RAEB, 11 CMML)</td>
<td>28</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>CLL</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myeloma</td>
<td>18</td>
<td>2 (2)</td>
<td>0</td>
</tr>
</tbody>
</table>

* No. of cases with 9p21 monosomy is shown in parentheses.

and another chromosome in 4 cases, i(9q) in 1 case, and del (9p) in 2 cases (del (9)p21) in 1 case, del (9)p13p21 in the other case). In 5 of those 7 cases, the rearrangement was associated to 9p21 monosomy, and therefore to probable hemizygosity for genes located on this band (including the p16 gene). In the other cases [the 2 cases of del (9p)], the breakpoint was in 9p21, and this did not allow us to determine if one allele of genes located in 9p21 band was lost.

Homzygous deletion of the p16 gene was seen in 9 cases (14%), including 6/39 (15%) B-precursor ALL, 3/12 (25%) T-ALL, and no Burkitt’s ALL (Table 3). In those 9 cases, the ratio between the hybridization signals from the p16 gene probe and the actin probe, measured by densitometry, was always less than 20% of that observed in controls (Fig 1).

In one of the patients with p16 gene homozygous deletion, cytogenetic analysis was a failure. Three of the 7 patients with detectable 9p rearrangement, including 3 of the 5 patients with 9p21 monosomy, had homozygous deletion of the p16 gene, compared to 5 of the 55 patients without detectable 9p abnormality.

Eight of the 9 patients (89%) with p16 gene homozygous deletion had one or several poor prognostic factors, including "bulky" disease in 8 cases, white blood cell (WBC) count >50 × 10^9/L in 7 cases, and "poor-risk" karyotype in 3 cases: t(9;22) (1 case), t(4;11) (1 case), and complex cytogenetic findings (1 case) (Table 3).

All 9 patients obtained complete remission (CR) with intensive chemotherapy but...
660  QUESNEL ET AL

Table 4. Hematologic Characteristics of the Patients Studied by PCR-SSCP of Exon 1 and Exon 2 of the p16 Gene

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Patients Studied</th>
<th>No. of Cases With 9p Rearrangement*</th>
<th>No. of Cases With Point Mutation of the p16 Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL (60 precursor B-ALL, 16 T-ALL, 12 Burkitt'ALL)</td>
<td>88</td>
<td>26 (12)</td>
<td>1 point mutation (5 polymorphisms at nucleotide 436)</td>
</tr>
<tr>
<td>AML (7 M1, 8 M2, 3 M3, 8 M4, 8 M5, 3 M6)</td>
<td>35</td>
<td>2 (2)</td>
<td>(3 polymorphisms at nucleotide 436)</td>
</tr>
<tr>
<td>MDS (7 RA, 12 RAEB, 11 CMML)</td>
<td>30</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>CLL</td>
<td>30</td>
<td>0</td>
<td>(2 polymorphisms at nucleotide 436)</td>
</tr>
<tr>
<td>Myeloma</td>
<td>30</td>
<td>2 (2)</td>
<td>(2 polymorphisms at nucleotide 436)</td>
</tr>
</tbody>
</table>

* No. of cases with 9p21 monosomy is shown in parentheses.

6 relapsed after 3 to 31 months. Median CR duration was 24 months, and median survival was 25 months (Table 3). By comparison, 44 of the 54 ALL (81%) without p16 gene homozygous deletion had one or several poor prognostic factors. Fifty-one of the 54 cases were treated with intensive chemotherapy and 38 (75%) achieved CR. Median CR duration was 18.5 months and median survival of the 51 patients treated intensively was 22 months. None of the differences between patients with and without p16 homozygous deletion were significant.

Southern Blot Analysis of IFNa Genes in ALL

Southern blots of the 63 ALL were rehybridized with the IFNa gene probe. Complete disappearance of one or several bands, suggesting homozygous deletion of at least part of the IFNa genes, was found in 3 cases (patients no. 30, 435, and 751), who also had homozygous deletion of the p16 gene (Fig 1). No homozygous deletion of IFNa genes was found in the S4 patients who had no homozygous deletion of the p16 gene.

PCR-SSCP Analysis of Exons 1 and 2 of the p16 Gene in ALL

PCR-SSCP analysis of exons 1 and 2 of the p16 gene (which cover 97% of the coding sequence) was performed in the 63 cases analyzed by Southern blot, and in 25 additional cases of ALL where DNA was obtained by scraping marrow smears from diagnosis samples. The 88 patients studied by PCR-SSCP included 6 children and 82 adults, 39 females and 49 males, 60 B-precursor ALL (58 early B-ALL and 2 pre B-ALL), 16 T-ALL, and 12 Burkitt's ALL (Table 4). Cytogenetic analysis (successfully performed in 87 cases) was normal in 23 cases. Four cases had a hyperdiploid karyotype, 6 had t(9;22), 4 had t(4;11), 2 had t(1;19), and the remaining patients had other variable anomalies. Twenty-six patients had a detectable 9p rearrangement, including: 12 cases of unbalanced translocation between chromosome 9 and another chromosome, 9p deletion or monosomy 9, all leading to 9p21 monosomy and therefore to loss of a p16 allele; 9 cases of del(9)(p21) or del(9)(p13)p21) where it could therefore not be determined if deletion of one p16 allele was present; and 5 balanced translocations between 9p21 and another chromosome, with apparently no material loss (Table 4).

Normal PCR-SSCP findings were observed in all 88 patients for exon 1 and in 82 cases for exon 2, by comparison with controls. Six patients had an abnormal SSCP profile for exon 2, which was identical in 5 of them, and differed in the remaining abnormal case (Fig 2). Direct sequencing of exon 2 showed that the 5 patients with similar abnormal SSCP findings had a G → A transition at nucleotide 436.

Fig 2. SSCP analysis of exon 2 of the p16 gene in 7 ALL cases. The amplified PCR product for exon 2 was migrated after digestion with Sma I enzyme (which separated it in two fragments). The 5' fragment, in case no. 157, had an abnormal migration, corresponding to a point mutation at nucleotide 164. The 3' fragment, in patient no. 1028, had an abnormal migration, corresponding to a probable polymorphism at nucleotide 436.
(codon 140, Ala → Thr). Persistence of normal wild-type bands by SSCP and sequence analysis, of approximately similar intensity as abnormal bands, showed that the mutation was heterozygous. The remaining patient had a C → T missense mutation at nucleotide 164, codon 49 (GCC → GTC) leading to Ala → Val substitution (Table 3). SSCP and sequence findings also showed that the mutation was heterozygous and that the remaining p16 allele was not mutated or deleted (Figs 2 and 3). This patient had B-precursor ALL, bulky disease, high WBC count, t(4;11) translocation, and a poor outcome. None of the patients with 9p rearrangement had detectable p16 gene mutation. Samples from the 9 patients with p16 gene homozygous deletion gave faint but normally migrating SSCP bands for exons 1 and 2, certainly corresponding to the amplification of DNA from residual marrow cells.

DNA from the 5 patients with the nucleotide 436 mutation was reanalyzed by PCR-SSCP after the patients had reached CR with combination chemotherapy. All 5 patients still had the SSCP profile observed at diagnosis, strongly suggesting that the G → A mutation at nucleotide 436 was a polymorphic variant.

**Southern Blot and PCR-SSCP Analysis of the p16 Gene in Other Hematologic Malignancies**

Eleven AML, 28 MDS, 20 CLL, and 18 myelomas were studied by Southern blot (Table 2). Those patients, and additional patients, leading to a total number of 35 AML, 30 MDS, 30 CLL, and 30 myelomas, also underwent PCR-SSCP analysis of exons 1 and 2 of the p16 gene (Table 4). All patients were successfully karyotyped and detectable rearrangements leading to 9p21 monosomy were only seen in 2 cases of AML, 1 case of MDS, and 2 cases of myeloma. Those patients had monosomy 9, and were studied by both Southern blot and SSCP.

No homozygous deletion of the p16 gene was found by Southern analysis in those patients. SSCP findings were normal for exon 1 in all cases, and for exon 2 in all but 7 cases. However, in those 7 cases which included 3 AML, 2 CLL, and 2 myelomas, the SSCP profile was identical to that observed in the ALL cases with nucleotide 436 G → A mutation. Direct sequencing, performed in two of them, confirmed the presence of this mutation, which probably corresponded to a polymorphism, as seen above.

**DISCUSSION**

This study represents, to our knowledge, the first analysis of deletions and point mutations of the p16 gene in uncultured samples from hematologic malignancies. Homozygous deletion was found in 14% of the ALL but in none of the 4 other malignancies studied, including AML, MDS, CLL, and myeloma. In ALL, homozygous deletion was not found in Burkitt's ALL, but was found in 15% of B-precursor ALL and 25% of T-ALL. Also of note was the homozygous deletion observed in HSB; and CEM cell lines, which are both derived from T-ALL.

Three of the 5 ALL cases with cytogenetic rearrangements leading to 9p21 monosomy and 5 of the 55 patients with cytogenetically normal 9p21 band had homozygous p16 gene deletion. This demonstrated submicroscopic deletion in band 9p21, involving one chromosome 9 in the former group, and both chromosomes 9 in the latter group. In the 9 deleted cases, no bands were seen by Southern analysis and, because the probe we used covered the entire coding region, it can be concluded that the entire p16 gene was deleted. In solid tumor cell lines with homozygous p16 gene deletion, the entire coding region was also generally deleted although, in a few cell lines, the deletion involved only exon 1 (or only exon 2). No abnormal band suggesting a chromosome 9 breakpoint within the p16 gene was seen, especially in the 2 ALL cases with a 9p21 breakpoint that were studied by Southern blot.

In primary solid tumors, the incidence of p16 gene homozygous deletions seems to be one third to one half of that observed in corresponding cell lines. These figures could also hold true for leukemias, because 55% of the 18 leukemia cell lines studied in two previous reports showed p16 gene homozygous deletion. However, no details on these cell lines were available to confirm if, as in uncultured leukemic samples, homozygous deletions predominated or were exclusively seen in ALL cell lines, by comparison with AML cell lines.

Using PCR-SSCP, a sensitive method for the detection of point mutations in DNA fragments no longer than 300 to 400 bp, we could find only one point mutation in exon 1 and 2 of the p16 gene (which cover 97% of the coding region of the gene) in 88 cases of ALL. This mutation was a missense mutation at nucleotide 164, codon 49, which had not been previously reported in tumors, to our knowledge. SSCP and sequence results showed that this mutation was heterozygous, the remaining p16 allele being still present and nonmutated. Five other cases of ALL had a G → A transition at nucleotide 436. This mutation had already been reported in 2 of 31 bladder cancers, 1 of 34 melanoma cell lines, and 4 of 75 various tumor types. In one of those reports, the mutation was also present in the patients' leukocytes. This nucleotide 436 G → A transition was also often
observed as a germline mutation in familial melanoma kindreds but, in those families, did not cosegregate with the disease, suggesting that it corresponded to a polymorphism. This conclusion was also supported by our findings, as the nucleotide 436 G → A transition was still present in the CR marmur of the 5 ALL cases that carried it on diagnosis samples. We also observed this probable polymorphism in 2 of the 30 myelomas, 3 of the 35 AML, and 2 of the 30 CLL analyzed by PCR-SSCP. In those 3 disorders, and in CLL, no other point mutations were seen.

Thus, only 1 of 88 ALL studied had a point mutation, although 12 of them had 9p21 monosomy, leading to probable loss of one p16 allele (and 9 had del 9p with a 9p21 breakpoint, possibly also associated to p16 allele loss in several cases). Loss of one gene copy is a situation where for tumor suppressor genes, a high incidence of point mutations inactivating the other allele is observed. Point mutations of the p16 gene, predominantly in exon 2, are seen in about one third of melanomas and pancreatic carcinomas but appear to be rare in the other solid tumors tested. In melanoma and pancreatic carcinoma, they are generally associated to loss of the normal residual allele. However, in the only case of ALL with a p16 point mutation reported here, cytogenetic, SSCP, and sequence findings showed that the normal residual p16 allele was still present. These findings suggest that, in ALL and in many solid tumor types (with the exception of melanoma and pancreatic adenocarcinoma), p16 gene inactivation mainly occurs through deletion of both copies of the gene, rather than by deletion of one allele and point mutation of the other allele, as often seen with the P53 gene.

In a previous report, Diaz et al showed that 7% and 22% of ALL had a homozygous and hemizygous deletion of interferon α genes, respectively, and that these deletions often occurred in the absence of detectable of deletion 9p21 band, where IFNα genes, like the p16 gene, are clustered. Because IFNα genes are situated very close to the p16 gene in 9p21, we also looked for homozygous deletions of these genes in our ALL cases. Only 3 of the 9 patients with p16 gene homozygous deletion and none of the 54 patients without p16 gene homozygous deletion had a homozygous deletion of one or several IFNα genes. This confirmed previous results in 14 leukemia cell lines, where 9 had a homozygous deletion of the p16 gene, and only 4 a homozygous deletion of IFNα 8 gene. Seven of those 14 cell lines had homozygous deletion of the methylthioadenosine phosphorylase (MTAP) gene, also situated close but in 5’ of the p16 gene. These findings suggest that, in ALL, homozygous loss of the p16 gene could have more pathogenetic importance than loss of neighboring genes (especially IFN and MTAP genes).

In this study we only focused on p16 gene homozygous deletions and did not determine the incidence of p16 gene hemizygous deletions. One of the reasons was that in our experience with Southern analysis, demonstrating of loss of only one copy of a gene in tumor cells contaminated by up to 20% or even 30% of normal residual cells is difficult. Furthermore, as for other tumor suppressor genes, inactivation of the p16 gene presumably requires the inactivation of both alleles, and the relevance of hemizygous deletions to the oncogenic process may be more hypothetical.

Finally, most of the ALL with p16 gene homozygous deletion or point mutations had one or several poor prognostic factors, including bulky disease and high WBC count, and most of them relapsed. However, these characteristics were not significantly different from those observed in patients without p16 homozygous deletion. Although larger numbers of cases of ALL with p16 gene homozygous deletion will be required before drawing conclusions, it does not appear that deletion of this gene is associated to specific hematologic and prognostic features in ALL.

In conclusion, our findings suggest that homozygous deletions of the p16 gene are seen in a small proportion of ALL, but is not observed or must be very rare in AML, MDS, CLL, and myeloma, whereas point mutations potentially inactivating p16 are very rare in those five disorders. In ALL, homozygous deletion does not appear to be associated to a specific immunophenotype and predominates but is not exclusively seen in ALL with 9p21 deletion. A pathogenetic role for p16 gene homozygous deletion in the development or progression of those ALL, suspected because of the potentially antiproliferative properties of p16, will have to be clearly shown.

REFERENCES


p16 gene homozygous deletions in acute lymphoblastic leukemia

B Quesnel, C Preudhomme, N Philippe, M Vanrumbeke, I Dervite, JL Lai, F Bauters, E Wattel and P Fenaux