Nonhereditary p53 Mutations in T-Cell Acute Lymphoblastic Leukemia Are Associated With the Relapse Phase

By Michael H. Hsiao, Alice L. Yu, Jo Yeargin, David Ku, and Martin Haas

We have previously reported that greater than 60% of human leukemic T-cell lines possess mutations in the p53 tumor suppressor gene. To determine whether T-cell acute lymphoblastic leukemia (T-ALL) patient samples possess p53 mutations, we screened peripheral blood- and bone marrow-derived leukemia samples, taken at diagnosis and at relapse, for p53 mutations. Exons 4 through 9 and selected intron regions of the p53 gene were analyzed using polymerase chain reaction–single-strand conformation polymorphism and direct sequencing, p53 mutations were found in 0 of 15 T-ALL diagnosis samples, as compared with 10 of 36 (28%) T-ALL relapse samples. To determine whether p53 mutations play a role in the recurrence (relapse) of T-ALL, two special groups of T-ALL patients were studied: (1) a group of 8 relapse patients whose disease was refractory to chemotherapeutic treatment, and (2) a group of 6 “paired” T-ALL cell samples from patients for whom we possess both diagnosis and relapse samples. Three of 8 relapsed patients (37.5%) whose disease was refractory to the reinduction of remission by chemotherapy possessed missense mutations of the p53 gene. All 3 cases had mutations in exon 5. Among the paired samples, 3 of 6 patients harbored p53 mutations at disease recurrence, but possessed only wild-type p53 alleles at diagnosis. One case had mutation on exon 4, 1 case in exon 5, and 1 case in exon 8 with loss of heterozygosity. These data clearly indicate that recurrence of T-ALL is associated with missense mutations in p53. Our results indicate that (1) mutations of p53 do occur in T-ALL in vivo, and such mutations are associated with the relapse phase of the disease; and (2) p53 mutation is involved in the progression of T-ALL. This conclusion is supported by our observation that the introduction of T-ALL-derived mutant p53 expression constructs into T-ALL cell lines further increases their growth rate in culture, enhances cell cloning in methylcellulose, and increases tumor formation in nude mice.

P53 belongs to the tumor suppressor class of genes whose function involves the negative regulation of cell growth. The loss of function of the p53 gene by deletion, mutation, or rearrangement may contribute to the genesis or progression of a wide variety of human cancers. Abnormalities in the p53 gene have been demonstrated in human carcinoma of the colorectum, lung, liver, bladder, and ovary; in blast crisis chronic myelogenous and myelocytic leukemias, and in progressions of a wide variety of human cancers. Abnormalities in the p53 gene have also been found in the precancerous phases of adenocarcinomas and in adenomatous polyps of patients with familial polyposis coli, suggesting that p53 mutation may occur as an early event in carcinogenesis as well.

In our studies on the role of p53 in the pathogenesis of human T-cell acute lymphoblastic leukemia (T-ALL), we have shown that greater than 60% of T-ALL cell lines, all of which had been grown from relapse T-ALL cases, possess mutations on both p53 alleles, suggesting that p53 serves a critical role in the generation of the fully tumorigenic leukemic T cells. However, our experiments did not show whether these p53 mutations originated in vivo or whether the p53 gene had been mutated during cell line establishment in vitro, as has been shown to occur during the establishment of some rat embryo fibroblast cell lines. Gaidano et al and Jonveaux and Berger have reported that samples from T-ALL patients obtained at diagnosis harbor no mutations in the p53 gene. In all, these two groups studied 37 T-ALL diagnosis samples, all of which possessed only wild-type p53 alleles. Recently, we reported that T-ALL cell lines established in our laboratory from a patient sample taken at relapse possess the same p53 mutation found in the in vivo sample. Furthermore, no additional mutations of p53 occurred during establishment of the cell lines. These results suggest that the establishment of human T-ALL cell lines need not be associated with the induction of p53 mutations.

To study whether p53 mutations may be differentially associated with the relapse versus diagnosis phases of T-ALL, we used polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) and direct sequencing of PCR-amplified fragments to study 15 cases of T-ALL at diagnosis and 36 cases at relapse. The results suggest that, in the patients studied, mutation of the p53 gene was associated with the clonal evolution that takes place during recurrence of the disease.

MATERIALS AND METHODS

T-ALL patient samples. Peripheral blood or bone marrow cells from 51 T-ALL patients at diagnosis or at relapse were donated in
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accordance with a protocol approved by the Committee on Investigation Involving Human Subjects at the University of California, San Diego. Some samples were kindly provided by the tumor cell bank of the Pediatric Oncology Group at St Jude’s Children’s Research Hospital. Peripheral blood or bone marrow cells were collected for the purpose of routine clinical diagnosis, and cells that remained after the diagnostic procedures were frozen live in liquid nitrogen for future use. High molecular weight DNA was extracted from one ampuole (10^7 cells) of each frozen T-ALL patient sample.

SSCP analysis. SSCP analysis was adapted from the original SSCP method of Orita et al.20 Briefly, 100 ng of genomic DNA was used in a PCR amplification. Each reaction mixture contained in addition to genomic DNA, 10 pmol of each primer, 2.5 μmol/L dNTPs, 1 μCi of [α-32P]dCTP (Amersham; specific activity, 3,000 Ci/mmol; 1 Ci = 37 GBq), 10 mmol/L Tris-HCl (pH 8.8), 50 mmol/L KCl, 1 mmol/L MgCl2, 0.01% gelatin, and 0.5 U of Taq polymerase, in a final volume of 10 μL. Thirty cycles of denaturation (93°C for 2 minutes), annealing (60°C for exons 4, 5, 5, and 8; 68°C for exon 7; 51°C for exon 9; for 2 minutes), and extension (72°C for 1 minute) were performed. Two microliters of the reaction mixture was diluted 25X in 0.1% sodium dodecyl sulfate (SDS)/10 mmol/L EDTA and further mixed 1:1 with a sequencing stop solution containing 20 mmol/L NaOH. Samples were heated to 95°C for 5 minutes and chilled on ice, and 3 μL was immediately loaded onto the gel. Gels were run at 4 W for 16 to 20 hours at room temperature. Autoradiography was performed with an intensifying screen for 6 to 24 hours. Because not all of the mutation bands were easily separated from normal germline bands with good resolution using one gel condition, three gel conditions were used for each sample: (1) 6% acrylamide with 10% vol/vol glycerol (better separation with poor resolution); (2) 4% MDE gel (AT Biochem, Malvern, PA) with glycerol (intermediate separation and resolution); and (3) 4% MDE without glycerol (best resolution with least separation). Mobility shifts that were detected by SSCP were confirmed under at least two different gel conditions, before sequencing.

The following p53 amplification primers were used:27 MH41 (5’ exon 4 primer), 5’-ATCTACAGTCCCCCTTGGCAC-3’; MH42 (3’ exon 4 primer), 5’-GCAAATGACCTGCAAGTCA-3’; MH22 (5’ exon 5 primer), 5’-CTGTTCATCTGGCCCTGAC-3’; MH20 (3’ exon 5 primer), 5’-CAACCGCCCTGTGCTCTC-3’; MH18 (5’ exon 6 primer), 5’-GAGAGCAGGAGGCTGGT-3’; MH29 (3’ exon 6 primer), 5’-CACTGACAAACACCCCCT-3’; MH30 (5’ exon 7 primer), 5’-CAAGGGCCACCTGCCTC-3’; MH31 (3’ exon 7 primer), 5’-GAGGCAAGCAGGACGTG-3’; MH19 (5’ exon 8 primer), 5’-GGGACACGTAGACCTGATT-3’; MH23 (3’ exon 8 primer), 5’-CAGCCTTCTGGCTCTCTT-3’; MH34 (5’ exon 9 primer), 5’-TAATGGCTTCCAGTCACTT-3’; MH25 (3’ exon 9 primer), 5’-CACTGAATTCTGGAATCCTCCACTGAT-3’.

Direct sequencing of PCR products. Samples that were found by SSCP analysis to possess p53 mutations were sequenced to identify the nature of the mutation. Solid-phase sequencing of in vitro amplified genomic DNA was used,27 in which genomic DNA was amplified by PCR using biotinylated primers (Operon, Almeda, CA). One microgram of genomic DNA was used as template in a 100 μL PCR reaction with 12 pmol of biotinylated primer and 36 pmol of nonbiotinylated primer. Forty microliters of this reaction was incubated with magnetic beads conjugated covalently with streptavidin (Dynabeads M280-streptavidin; Dynal, Oslo, Norway) that were used to selectively immobilize the biotin-labeled PCR product and allow melting of the DNA duplex, followed by elution of the nonlabeled single strand. The immobilized single-strand DNA was then used as sequencing template using the Sequenase (US Biochemical Co, Cleveland, OH) protocol and an internal primer.

RESULTS

p53 mutation is a frequent genetic change in relapse T-ALL. We used PCR-SSCP analysis to screen for p53 muta-

tions in the genomic DNA of 51 patients with childhood acute lymphoblastic leukemia at different clinical stages (15 diagnosis T-ALL, 36 relapse T-ALL). Significant electrophoretic mobility shifts were detected in 10 of the 36 relapse T-ALL samples (28%), and in none (0 of 15) of the diagnosis T-ALL samples (0%). Figure 1 shows examples of PCR-SSCP analyses of exons 4 and 5. DNA extracted from a normal human placenta was used as normal control to show the germline bands. The T-ALL cell line CEM, which harbors a mutation in p53 codon 175,20 was used as a mutant control of exon 5 to show different mobility shift patterns under two different gel conditions (see legend to Fig 1 and Materials and Methods).

Direct sequence analysis was performed on the 10 samples found to be positive for p53 mutations based on significant SSCP shifts. Most of the mutations found were missense mutations with single-base changes that encoded single amino acid substitutions in the p53 protein (Table 1). The majority of the mutations found are located in exon 5. One patient, PJN, had an intron 4 mutation. Although its biologic significance has not been studied further, its detection shows that cells harboring this mutation were selected for; hence,
Table 1. Frequency and Nature of p53 Mutations in Primary T-ALL Samples

<table>
<thead>
<tr>
<th>T-ALL Patient Sample</th>
<th>SSCP Codon</th>
<th>Seq Change</th>
<th>aa Change</th>
<th>Genotype</th>
<th>% Blasts</th>
</tr>
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<tbody>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/15 (0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapse 10/36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(28%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMN</td>
<td>Ex 4 110</td>
<td>CGT → CTT</td>
<td>Arg → Leu</td>
<td>Heterozygous</td>
<td>95</td>
</tr>
<tr>
<td>FAN</td>
<td>Ex 5 135</td>
<td>TGC → AGC</td>
<td>Cys → Ser</td>
<td>Heterozygous</td>
<td>92</td>
</tr>
<tr>
<td>ONN</td>
<td>Ex 5 135</td>
<td>TGC → AGC</td>
<td>Cys → Ser</td>
<td>Heterozygous</td>
<td>90</td>
</tr>
<tr>
<td>ASN</td>
<td>Ex 5 135</td>
<td>TGC → TAC</td>
<td>Cys → Tyr</td>
<td>Homozygous*</td>
<td></td>
</tr>
<tr>
<td>GCN</td>
<td>Ex 5 141</td>
<td>TGC → TCT</td>
<td>Cys → Phe</td>
<td>Heterozygous</td>
<td>91</td>
</tr>
<tr>
<td>SRN</td>
<td>Ex 5 161</td>
<td>GCC → ACC</td>
<td>Ala → Thr</td>
<td>Heterozygous</td>
<td>90</td>
</tr>
<tr>
<td>FMN</td>
<td>Ex 5 167</td>
<td>CAG → CGG</td>
<td>Gln → Arg</td>
<td>Homozygous*</td>
<td></td>
</tr>
<tr>
<td>HAN</td>
<td>Ex 5 175</td>
<td>ACG → ATG</td>
<td>Thr → Met</td>
<td>Homozygous*</td>
<td></td>
</tr>
<tr>
<td>BTN</td>
<td>Ex 8 282</td>
<td>CGG → GGG</td>
<td>Arg → Gly</td>
<td>Heterozygous</td>
<td>93</td>
</tr>
<tr>
<td>PJN</td>
<td>Intron 4</td>
<td>C → T</td>
<td></td>
<td>Homozygous†</td>
<td></td>
</tr>
</tbody>
</table>

* Wild-type allele at this locus undetectable on sequencing gel, but no LOH by codon 72 polymorphism (see Fig 7).
† Homozygous mutation verified by LOH determination (see Fig 7).
‡ C → T mutation occurs in intron 4 at position 13049 (Ref: Genebank sequence accession no. X54156).

this intronic mutation may possess biologic significance. Figures 2 and 3 show representative examples of the direct
sequencing data. Figure 2 demonstrates two relapse T-ALL samples (GCN and HAN, respectively) with heterozygous
missense mutations in exon 5. Interestingly, three separate
cases possess mutations at codon 135. Two of these cases
(FAN and ONN) possess a heterozygous mutation with nu-
cleotide change from TGC to A/TGC (135YY6), whereas
a homozygous mutation with nucleotide change from TGC to
TAC (135YYs) was found in the peripheral blood-derived
leukemic cells of patient ASN (Fig 3).

The apparent heterozygous nature of the mutations sum-
marized in Table 1 may be caused by contamination of the
leukemia samples with normal cells. Table 1 shows that at
the time of DNA extraction the percent blast cells present
in the patients’ cell samples was at least 90%. The minority
(wild-type) alleles detected could not have been caused by
contamination with normal cells at the less than 10% level,
because in a mixture of mutant and wild-type DNA mole-
cules the minority component is detectable on SSCP or se-
quencing gels only when present at the 20% level or higher.†
Hence, we conclude that the heterozygous nature of the mu-
tations presented in Table 1 and in the corresponding figures
represent mutations in one allele of the leukemia cells, whereas
the other allele remains wild-type. To verify this
conclusion, we have tested whether unrearranged (ie, germ-
line) T-cell receptor (TCR) β-chain DNA could be demon-
strated in the samples tested. Southern blots containing
BamHI-digested genomic T-ALL DNA were hybridized
with a probe specific for the human Tβ locus. All 6 samples
showed rearranged TCRβ bands, whereas in only one (ONN)
patient sample could a band of apparent germline size be
shown (data not shown). Hence, the p53 mutations shown
in Table 1 represent bona fide heterozygous mutations that
are not caused by contamination of the leukemia cell samples
with normal cells.

![EXON 5](image-url)
p53 mutation is somatically acquired and is associated with the recurrence of T-ALL. Ten of 36 relapse T-ALL cases studied (28%) possess p53 mutations in the p53 gene. Because these are random T-ALL patient samples collected during a 5-year period, there is no means of determining whether the mutations found are germline or somatically acquired, nor what role, if any, p53 mutations play in the recurrence of T-ALL. To determine the pathologic significance of p53 mutations in the recurrence (relapse) of T-ALL, a group of 6 “paired” T-ALL cell samples from patients for whom we possess both diagnosis and relapse samples were studied. PCR-SSCP and direct sequencing showed that none of the samples taken at diagnosis possessed p53 mutations, whereas 3 of 6 (50%) of the “paired” T-ALL samples studied harbored missense mutations at the relapse phase of the disease (Table 2). Figure 4 shows that a mutation was present in the leukemic cells of patient SMN at relapse. This mutation was absent from SMN’s leukemic cells at diagnosis. PCR-direct sequencing data identify the nature of this mutation as a heterozygous missense mutation with a nucleotide change from CGT to CTT (110Arg → Leu). A heterozygous missense mutation with a nucleotide change from GCC to ACC (161Ala → Thr) was found in the leukemic cells of patient SRN at relapse but not at diagnosis (Fig 5). Furthermore, a homozygous missense mutation was detected in the leukemic cells of patient BTN at relapse but not at diagnosis. Figure 6 shows this mutation as found by SSCP (arrow). Direct sequencing data establish the nucleotide change at relapse, a codon change from CGG to GGG (282Arg → Gly).

We also studied whether p53 mutations were present in the cells of a group of 8 T-ALL relapse patients whose

<table>
<thead>
<tr>
<th>Table 2. p53 Mutations in Paired Diagnosis/Relapse T-ALL Patient Samples</th>
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<tbody>
<tr>
<td>Patient</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>AHN/D</td>
</tr>
<tr>
<td>AHN/R</td>
</tr>
<tr>
<td>BTN/D</td>
</tr>
<tr>
<td>BTN/R</td>
</tr>
<tr>
<td>LNN/D</td>
</tr>
<tr>
<td>LRN/R</td>
</tr>
<tr>
<td>SZN/D</td>
</tr>
<tr>
<td>SZN/R</td>
</tr>
<tr>
<td>SRN/D</td>
</tr>
<tr>
<td>SRN/R</td>
</tr>
<tr>
<td>SMN/D</td>
</tr>
<tr>
<td>SMN/R</td>
</tr>
</tbody>
</table>

Abbreviations: /D, patient sample obtained at diagnosis; /R, patient sample obtained at relapse.
*Homozygous/heterozygous mutations, genotype verified by codon 72 polymorphism (see Fig 7).
disease was refractory to chemotherapeutic treatment.30,31 p53 exons 4 through 9 were screened for mutations using PCR-SSCP and direct sequencing. Three of 8 patients (37.5%) in this group possess p53 mutations as detected by PCR-SSCP. The p53 mutations in the group of refractory T-ALL cases all mapped to exon 5. Direct sequencing showed a homozygous missense mutation in codon 135 with a nucleotide change from TGC to TAC (135cys'Tyr) in patient ASN (Table 3). Leukemic cells of patients SRN and HAN harbored heterozygous missense mutations in codon 161 (GCC to ACC, 161Ala'Thr) and in codon 175 (CGC to GGC, 175Arg'Cys), respectively (Table 3). Taken together, the presence of p53 mutations in 33% to 50% of relapse T-ALL cells in the different groups of patient samples studied suggests that p53 gene mutation is associated with the tumorigenic progression of relapse T-ALL cells.

Loss of heterozygosity at the p53 locus is an infrequent event in relapse T-ALL. In human solid tumors, mutations of the p53 gene are frequently accompanied by the loss of heterozygosity of chromosome 17p13.1, the locus to which the p53 gene maps. We have previously reported that, in leukemia cell lines, both alleles of the p53 gene were often independently mutated without concomitant loss of heterozygosity at the 17p13.1 locus. To investigate whether loss of heterozygosity of p53 is a frequent event during recurrence of T-ALL, we used the known polymorphism at codon 72 of the human p53. This polymorphism is encoded by sequences in exon 4 of the p53 gene. The frequency of the 72GAG- and the 72CGC-encoding alleles (CGC and CCC, respectively) in the human population is 0.68 and 0.32, respectively.36 Thus, 44% of the human population are heterozygous at this locus, whereas 46% and 10% encode homozy-
Fig 6. SSCP analysis and direct sequencing of mutations in exon 8 of “paired” diagnosis/relapse T-ALL samples. Human placenta DNA was used as a normal control (not shown) and DNA extracted from the breast carcinoma line MDA-MB-231 (HTB-26), which carries a mutation in p53 exon 8, was used as a positive control for SSCP. Gel condition for SSCP: 4% MDE gel.

Table 3. p53 Mutation in T-ALL Cases Refractory to Chemotherapy

<table>
<thead>
<tr>
<th>Identification of Mutation</th>
<th>Sequencing</th>
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<tbody>
<tr>
<td>Patient</td>
<td>SSCP (exon)</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>ASN</td>
<td>4</td>
</tr>
<tr>
<td>CJN</td>
<td>5</td>
</tr>
<tr>
<td>GENTI</td>
<td>6</td>
</tr>
<tr>
<td>HAN</td>
<td>7</td>
</tr>
<tr>
<td>HJN</td>
<td>8</td>
</tr>
<tr>
<td>HMN</td>
<td>9</td>
</tr>
<tr>
<td>JRN</td>
<td>4</td>
</tr>
<tr>
<td>SRN</td>
<td>5</td>
</tr>
</tbody>
</table>

* Homozygous/heterozygous mutations, verified by status of codon 72 polymorphism.
† This patient’s leukemia was refractory to chemotherapy, but relapse was not formally established.
antigen binding region of the p53 protein,38 suggesting that the mutations were biologically significant by conveying a selective growth advantage to the cells so endowed. In recent work (M. Hsiao, E. Dorn, J. Yeargin, and M. Haas, manuscript submitted), we have observed that mutated p53 genes that had been cloned from relapse T-ALL cells and constructed into retroviral expression vectors indeed possess dominant oncogenic activity when introduced into T-ALL cells, as assayed by in vitro and in vivo criteria. Thus, p53 dominant oncogenic activity when introduced into T-ALL diagnosis samples lacked detectable p53 mutations in exons course, were studied for the status of the p53 gene. All six doubt, that mutation of the p53 gene in relapse samples is evaluable polymorphic cases displayed the mutations were biologically significant by conveying a selective growth advantage to the cells so endowed. In recent work (M. Hsiao, E. Dorn, J. Yeargin, and M. Haas, manuscript submitted), we have observed that mutated p53 genes that had been cloned from relapse T-ALL cells and constructed into retroviral expression vectors indeed possess dominant oncogenic activity when introduced into T-ALL cells, as assayed by in vitro and in vivo criteria. Thus, p53 mutations in relapse T-ALL cells most probably play a biologically significant role in the disease.

The lack of p53 mutations in any of the “diagnosis” samples suggests, but does not prove beyond a reasonable doubt, that mutation of the p53 gene in relapse samples is specifically associated with the progression of the leukemic phenotype. To prove that p53 mutations in relapse leukemia represent clonal evolution events, we compared the status of the p53 gene in diagnosis and in relapse samples in a pairwise fashion. Six pairs of leukemia cell samples, each derived from the same patients at different points in the clinical course, were studied for the status of the p53 gene. All six diagnosis samples lacked detectable p53 mutations in exons 4 through 9, whereas in 3 of the 6 relapse samples (50%) a mutation had occurred in the p53 gene (Table 2). Clearly, p53 mutation is associated with the clonal evolution of relapse leukemia in a significant fraction of cases. This result supports our previous finding that a significant fraction of acute leukemia cell lines, all derived from relapse cases, possess mutations in the p53 gene.

In some human solid tumors mutation of one p53 allele is often accompanied by the loss of the other p53 allele.16,39 However, in acute leukemia cell lines loss of heterozygosity is an infrequent finding,23 and in the acute leukemia patient samples reported here, only 1 patient (BTN) among the 47 evaluable polymorphic cases displayed loss of heterozygosity of the p53 locus, as determined by the status of the polymorphic marker at codon 72. Although in greater than 50% of acute leukemia cell lines each p53 allele carried an independent mutation, in the current set of patient samples only 1 relapse case possessed two independent mutations (FMN, Table 1). These two mutations occurred on the same allele, as determined by molecular cloning (not shown). This discrepancy between leukemia cell lines and patient relapse samples suggests either that relapse leukemia cells possessing independent mutations in both alleles are favored during establishment in vitro, or that, in the lines, the second allele became mutated upon establishment.

Because the failure of chemotherapeutic treatment of acute childhood leukemia is often caused by the development of a drug-resistant clone, acute leukemias of patients who are refractory to the induction of remission might possess mutated p53 genes, as suggested by work of Cabanillas et al30 and Chin et al.31 To test the possible correlation between refractoriness to remission induction and p53 mutation in acute T-cell leukemia, we tested a set of 8 refractory cases for the status of p53. Three of the 8 cases (37%) possess a mutation in the p53 gene (Table 3). Interestingly, in these 3 refractory cases, the mutations were all located in exon 5, a hotspot of p53 mutations in human cancer.40 The possibility that these exon 5 mutations specifically affect the drug-resistant status of the leukemia cells raises interesting questions.

In each of the groups of relapse leukemia cases examined, the incidence of cases found to harbor p53 mutations probably represents a lower limit. Both the SSCP and the direct sequencing techniques fail to detect mutations that are present in less than 20% of the cell populations.41 In addition, mutations may be present in exons other than exons 4 through 9 examined, or in introns. Alternatively, the frequency of mutations that we have found in relapse leukemias may represent only a fraction of the cases in which the wild-type suppressor function of p53 is inactivated. Relapse cases that lack detectable p53 mutations may have lost some of the p53 functions by inactivation of the p53 protein through complex formation with other cellular proteins, eg, mdm-2.42,43 Studies of the inactivation of p53 function in relapse leukemias by means other than mutations are in progress.

It is important to consider why acute leukemia patient samples as well as cell lines infrequently display LOH at the p53 locus, whereas human solid tumors often show LOH at the p53 locus on chromosome 17p. The difference may
lie in the nature of acute leukemia cells as compared with cells of malignant solid tumors. The generation of malignant human solid tumors requires a series of 6 to 8 or more genetic alterations in the same cell; the malignant cells have undergone fewer genetic alterations and that have few, if any, characteristics of "tumor cells." Specifically, acute leukemia cells are "malignant," ie, life-threatening, even though they merely display the characteristics of differentiating normal precursor cells (ibid), and may lack many or most of the in vitro criteria of "tumor cells." We propose that, in acute leukemia cells, mutation of each p53 allele provides the leukemic cell with a growth advantage through the elimination of normal suppressor functions (M. Hsiao, E. Dorn, I. Yeargin, and M. Haas, manuscript submitted, and Dittmer et al). Thus, whereas the acquisition of each dominantly acting mutated p53 allele is significant for the potentiation of the leukemic phenotype of acute leukemia cells, in solid tumors a fully tumorigenic and metastatic phenotype necessitates the loss of each functional p53 allele, hence the frequent loss of the functional allele through LOH.

REFERENCES

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Nonhereditary p53 mutations in T-cell acute lymphoblastic leukemia are associated with the relapse phase

MH Hsiao, AL Yu, J Yeargin, D Ku and M Haas