N-Ras Gene Point Mutations in Childhood Acute Lymphocytic Leukemia Correlate With a Poor Prognosis


Ras genes can be altered by point mutations at critical portions of their coding regions to acquire transforming ability in vitro. These point mutations have been detected in a variety of human malignancies. However, their relevance for the clinical and biologic behavior of the subgroups of patients exhibiting these mutations is unclear. We analyzed 100 patients with childhood acute lymphocytic leukemias (ALLs) for point mutations of exons 1 and 2 of all three ras genes (H-ras, K-ras, and N-ras) by polymerase chain reaction and a combination of oligonucleotide hybridization and direct DNA sequencing. A 6% incidence of N-ras gene mutations was detected, all of which occurred at different nucleotides of codons 12 or 13 of N-ras.

A LARGE BODY of knowledge indicates that cellular transformation in human malignancies is caused by a combination of genetic events, at least one of which may involve activation of a protooncogene. Of these genes, members of the ras gene family (H-ras, K-ras, N-ras) have been extensively characterized on the molecular and biochemical levels. The ras genes code for highly homologous 21-Kd guanosine triphosphate (GTP)-binding proteins (p21) with intrinsic GTPase activity that are located at the inner cellular membrane. These features of the ras proteins suggest that they are related to the so-called "G-proteins"; thus, their functions may lie in transduction of receptor-mediated extracellular signals into the cell. Certain structural alterations in ras proteins lead to loss of GTPase activity and conceivably to the subsequent inability to "switch off" signals. These alterations can be produced by point mutations in distinct domains of the coding regions of all three ras genes. An "activated" ras gene carrying a point mutation of codon 12, 13, or 61 has transforming ability in several biologic assays.

Altered ras genes have been detected with varying frequencies in a number of human malignancies. N-ras mutations are found in several types of leukemias; in a number of large studies, their occurrence in acute myelogenous leukemia (AML) varies between 25% and 40%. The presence of ras mutations may be of clinical importance if they correlate with biologic features or clinical outcome. To date, no large study has examined the incidence or type of ras gene mutations in different forms of childhood acute lymphocytic leukemia (ALL). A previous report has found N-ras mutations at the first nucleotide of codon 12 in 2 of 19 children with ALL.

Primer-directed in vitro amplification by polymerase chain reaction (PCR) of ras genes combined with oligonucleotide hybridization provides a fast and sensitive screening assay for these mutations. Using this approach, we examined DNA from 100 patients with childhood ALLs for point mutations at critical regions of exons 1 and 2 of all three ras genes, and correlated the results with the biologic features and clinical outcome of these cases.

When correlating presence of ras mutations with the clinical and biologic features and the clinical outcome of these cases, a significantly higher risk for hematologic relapse (P = .01) and a trend toward a lower rate of complete remission (P = .07) was noted. The two groups did not differ in any of the known high-risk factors of ALL. These results suggest that presence of an N-ras mutation in children with ALL may be an independent predictor for worse clinical outcome and therefore may have therapeutic implications; further studies to confirm these findings are required because of the small number of patients with N-ras mutations.

MATERIALS AND METHODS

Patients. All investigations were approved by the St Jude Children's Research Hospital Clinical Trials Committee (Memphis, TN); informed consent for all studies and therapy was obtained from each patient or their parents as appropriate. The diagnosis of ALL was based on the morphologic and cytochemical criteria of the French-American-British (FAB) Cooperative Working Group. All cases had fewer than 3% blasts positive for myeloperoxidase, Sudan Black B, or α-naphthyl butyrate esterase. Bone marrow samples were obtained before any anti-leukemic therapy, and were separated on a Ficoll-Hypaque gradient (1.077 g/cm³). In all cases, 85% or more of the cells in each sample were leukemic blasts by morphology. These 100 cases had a minimal follow-up of 1 year in complete remission and were selected at random from children treated between January 3, 1984 and October 24, 1987. All patients in this series were treated on a single clinical therapy trial (Total XI), the details of which have been previously reported.

Cells. Normal lymphocytes were isolated from peripheral blood of healthy donors. As controls in the detection of ras mutations, cell lines with known ras mutations were used: MOLT-4 (T-lymphoblastic, mutation at position 1 of codon 12 [N12p2] of N-ras); PA-1 (teratocarcinoma cell line, mutation at N12p2); HL-60 (promyelo-
cytic, mutation at N61p2); 44-911 NIH 3T3 cells (murine fibroblasts transformed by a human H-ras gene with a mutation of H12p2); 118-413 NIH 3T3 cells (murine fibroblasts transformed by a human K-ras gene mutated at K12p124 (NIH 3T3 cell lines were generous gifts of M. Barbacid, Frederick Cancer Research Facility, NCI, Frederick, MD). Cells were grown in a medium (Flow Laboratories, McLean, VA) supplemented with 10% fetal calf serum (FCS; Irvine Scientific, Santa Ana, CA) in a 7.5% CO2 humidified atmosphere.

Immunophenotyping. Immunophenotyping was performed by a standard indirect immunofluorescence assay using a panel of monoclonal antibodies (MoAbs) including CD2, CD3, CD7, CD10, CD13, CD15, CD19, and CD33. Results were considered positive if more than 25% of the cells expressed the antigen (isotopically matched myeloma immunoglobulins at the same concentration were used as negative controls). Leukemic cells were also tested for cytoplasmic immunoglobulin (cIg). Based on reactivity, cases were classified as T-ALL (CD2+ and CD7+); early B-precursor ALL (CD19+, CD10-, cIg-); common ALL (CD19+, CD10+, cIg-), or pre-B ALL (cIg+). Cases expressing any myeloid-associated antigen were described as mixed-phenotype ALL.

In vitro amplification of ras genes by PCR and blotting of PCR product. DNAs were isolated from leukemic cells from 100 children and from cell lines as previously described.25.26 High molecular weight genomic DNA (500 ng) was amplified for exons 1 and 2 of all three ras genes by symmetrical PCR essentially as described by Saiki et al13 (sequences of amplimers available on request). Twenty-five cycles of PCR reaction were performed in a DNA Thermal Cycler using Taq DNA polymerase (Perkin-Elmer/Cetus Corp, Emeryville, CA). Amplified product (5 µL) was electrophoresed on duplicate NuSieve/agarose gels (3%/1%, FMC Bioproducts, Rockland, ME) with Tris-borate buffer. After electrophoresis, DNA was transferred to nylon membrane (Zetaprobe, Bio-Rad Laboratories, Richmond, CA) according to Reed and Mann.27 After completion of transfer, filters were rinsed in 2X SSPE (1X SSPE = 10 mmol/L sodium chloride, 0.18 mol/L NaCl, 1 mmol/L EDTA, pH 7.0) at room temperature and air-dried.

Oligonucleotide probe hybridization. Filters were prehybridized for 2 hours (for exon 1 probes of N-ras and K-ras at 63°C; exon 1 of H-ras at 67°C; exon 2 of N-ras and K-ras at 59°C; exon 2 of H-ras at 63°C) in the presence of 5X SSPE, 5X Denhardt's solution, 100 mg/mL denatured sonicated salmon sperm DNA, 10 mmol/L EDTA, and 1% sodium dodecyl sulfate (SDS). Hybridization was performed for 2 to 4 hours under the same conditions but in the presence of the respective oligonucleotide probe (2 x 10^6 cpm/mL hybridization solution) end-labeled with [γ-32P]adenosine 5' triphosphate (ATP) using T4 kinase (New England Biolabs, Beverly, MA). For detection of the mutations, mixtures of oligonucleotide probes, which allow detection of each of all possible activating base pair (bp) substitutions at a given nucleotide position, were used (sequences of probes available on request). After hybridization, high-stringency rinses were performed in 5X SSPE, 0.1% SDS for 5 to 20 minutes at either 63°C (probes for exon 1 of N-ras, K-ras, H-ras), 72°C (exon 1 of H-ras), 59°C (all probes for exon 2 of N-ras and K-ras), or 67°C (exon 2 H-ras). Filters were exposed to Kodak X-AR 5 film (Rochester, NY) for 3 to 24 hours at -70°C with intensifying screens. Before rehybridization, bound probe was removed by rinsing at 65°C for 15 minutes in 0.1X SSPE.

Dideoxy-sequencing of in vitro amplified DNA. Amplified DNA was gel-purified, and in a modification of the 3-primer method, 16 ng of amplified template DNA was annealed with an equimolar amount of one of the ampilmer oligonucleotides (5'-end-labeled with [γ-32P]ATP). Annealing mixture was heated to 95°C for 5 minutes and chilled on ice for 10 minutes; 1 µL of 0.1 mol/L dithiothreitol (DTT) and 8 U modified T7 DNA polymerase (Sequenase, US Biochemical Corp, Cleveland, OH) were added. Reaction mixture was appor- tioned into four tubes containing deoxy- and dideoxynucleotides (10:1). Chain termination28 took place at 45°C for 10 minutes. The reaction was terminated by addition of formamide/dye stop mix; samples were heated to 95°C for 3 minutes, chilled, and electrophoresed on a 10% polyacrylamide/7M urea gel at 50°C for 4 hours. Gels were dried and exposed to Kodak X-AR 5 film at -70°C with an intensifying screen.

Statistical analysis. Differences in the distribution of clinical and biologic features among subgroups of patients were tested by the Fisher's exact test (2-tailed). Wilcoxon rank series test was used to examine differences between continuous variables. The clinical and biologic features analyzed were: age at diagnosis, sex, presence of central nervous system (CNS) disease, presence of a medistinal mass, hepatomegaly, splenomegaly (greater than 5 cm below the costal margin), leucocyte and platelet counts, hemoglobin, FAB classification, immunophenotype, DNA index, number of chromosomes, and presence of chromosomal translocation. Time to relapse and time to failure curves were calculated with the Kaplan-Meier technique.29 Differences in these curves were tested with log-rank statistics.

RESULTS

Point mutations at codons 12 or 13 of N-ras in childhood ALL correlate with prognosis. In the initial screening, DNAs from leukemic blasts from 100 patients with different forms of childhood ALL were amplified for exons 1 and 2 of all three ras genes by PCR using heat-stable Taq DNA polymerase. The amplified product was hybridized to an array of probes recognizing a total of 48 different possible point mutations. Figure 1 shows a composite representing the results obtained from the analysis of N-ras exon 1 in all patients. Amplified DNAs were electrophoresed (Fig 1A), ethidium-bromide stained gel, blotted to nylon filters, and hybridized to a probe complementary to unmutated ("wild-type") N-ras exon 1 sequences (Fig 1B). The filters were sequentially hybridized to four probe mixtures complementary to all possible bp substitutions of positions 1 and 2 of codons 12 and 13 of N-ras (Fig 1C). Hybridization and rinsing conditions were chosen at which only fully matched hybrids were thermally stable, but a single bp mismatch would prevent hybridization. Conditions were monitored by using DNA from cells with known ras mutations as controls (indicated by arrows on Fig 1C). All mutations identified by the first screening were confirmed by repeated, independent amplification and hybridization experiments.

The immunophenotypic classification of the 100 children with ALL was early B-precursor cell ALL (n = 15), common ALL (CALLA-; n = 44), pre-B ALL (n = 19), and T-ALL (n = 20). Only two patients’ blasts also expressed myeloid-associated antigens. Therefore, by standard immunophenotype classification, 80 cases were B-lineage and 20 cases were T-lineage ALL. In leukemic blasts from a total of 6 of 100 ALL patients, mutated sequences were prevalent that involved either codon 12 or 13 of N-ras exon 1 (Fig 1C, Table 1). Five of the patients with ras mutations had B-precursor cell ALL (two early B-precursor; three CALLA-) and one patient had T-ALL. Cells from all of the patients also contained unmutated ("wild-type") N-ras sequences (Fig 1B). Screening of N-ras codon 61 (exon 2) and codons 12 and 61 of both K-ras and H-ras genes showed no
mutations in any of the 100 samples (data not shown). Three patients with N-ras mutations were tested both at diagnosis and while in complete remission. Analysis of the remission samples showed disappearance of the mutations in all three cases (Figs 1C and 2).

No apparent clinical or biologic characteristics were significantly different for the patients with N-ras gene mutations when compared with those without N-ras mutations (Table 2). The mean age at diagnosis, as well as the number of patients in the high-risk categories with an age less than 2 or greater than 10 years, were not different between these subgroups. No difference in sex, race, liver, or spleen size was noted in these two groups (Table 2). No difference was found in either the leukocyte count at diagnosis or the percentage of patients presenting with the high-risk feature of a leukocyte count >25 x 10^9/L. Likewise, the hemoglobin, platelet count, FAB classification, and T-cell immunophenotype were not significantly different. The percentage of patients with hyperdiploid leukemia or chromosomal translocations were identical in the two subgroups. Thus, although the number of patients in the group with ras gene mutations was small, the clinical and biologic features known to predict a poor response to therapy were identical to the patients without N-ras mutations.

To determine whether the ras gene mutations had any correlation with clinical outcome, the patients with ras mutations were compared with those without mutations. Hematologic relapse was significantly more frequent in the ALL patients with ras gene mutations (P = .01), Table 3; Fig 3). When all types of relapse (hematologic and CNS) were considered, a trend toward a lower rate of complete

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**Fig 1.** Mutations of exon 1 of the N-ras gene in childhood ALL detected by oligonucleotide hybridization. The figure shows all mutations detected in this study. DNA from cells of 46 patients with newly diagnosed childhood ALL, from remission samples of three of these patients, and of five patients with newly diagnosed childhood AML were examined by PCR and oligonucleotide hybridization. DNA from normal human peripheral blood lymphocytes (PBL), from cell lines with known N-ras gene mutations, from a patient with adult AML carrying a known mutation at N13p2 (Lübbert M, Miller CW, Koehler HP; unpublished result, May 1988) were used as controls for the hybridization assay (indicated by arrows in C). Genomic DNA, 0.5 μg, was amplified in vitro as described in Materials and Methods. One tenth of the total reaction volume (≈ 5 μL) was electrophoresed. The ethidium-bromide stained gel is shown in panel (A). DNA was transferred to nylon-based membrane (Zetaprobe, Bio-Rad) and sequentially hybridized to an oligonucleotide probe complementary to wild-type N-ras exon 1 sequences (B), and to each of four probe mixtures complementary to all possible mutations at the first and second nucleotides of N-ras codons 12 and 13 (C). Filter was rinsed at the conditions described in Materials and Methods and autoradiographed for 3 to 8 hours. Upper row (a): lane 1, PBL from normal donor; lane 2, MOLT-4 (N12p1 mutation); lane 3, PA-1 (N12p2 mutation); lane 4, patient with adult AML (N13p1 mutation); lanes 5 through 29, ALL patients sampled at time of diagnosis; lane 30, 6X 174 RF DNA cut with Haelll. Lower row (b): lanes 1 through 21, ALL patients sampled at time of diagnosis; lanes 22 through 24, ALL patients sampled in remission; lanes 25 through 29, childhood MDS patients sampled at diagnosis (manuscript in preparation); lane 30, 6X 174 RF DNA cut with Haelll. For identification of ALL patients with mutations, see Table 1. Patients not shown in this figure did not exhibit N-ras point mutations.
finding must be interpreted with caution because of the small number of patients with ras gene mutations. The overall time to failure indicated that no significant difference existed between the two groups (P = .19).

Analysis of N-ras mutations in patients with ALL by direct DNA sequencing. In vitro amplified DNA from two of the patients was directly sequenced to confirm presence of the N-ras mutations by a second assay and to determine not only the nucleotide position but also the nucleotide transition and subsequent amino acid substitution of the mutation. Our protocol used one of the amplification primers as sequencing primer. Because the only ras mutations previously described in childhood leukemia involved codon 12 of N-ras, we were interested in further characterizing a mutation at codon 13. DNA from patient J.P. (early B-precursor cell ALL), who showed strongest predominance of this type of mutation by oligonucleotide hybridization assay, was amplified for exon 1 of N-ras. The PCR-product was sequenced by the method of Sanger et al\textsuperscript{29} using the antisense amplification primer. As shown in Fig 4A, position 1 of codon 13 contains both cytidine (wild-type sequence) and an additional adenine deoxynucleotide. This result was confirmed by also sequencing the sense strand (result not shown). This mutation led to a glycine-to-cysteine amino acid substitution resulting in a 10.6 protein with known transforming ability in vitro.\textsuperscript{12} In a similar manner, a mutation of N-ras codon 12 was characterized in patient S.F. (early B-precursor cell ALL). As shown in Fig 4B, DNA sequencing showed a thymidine in addition to the cytidine nucleotide (wild-type sequence) at position 1 of codon 12. This mutation led to a change in the coding sequence from glycine to serine.

**Table 1. Patients With Childhood Leukemias Exhibiting N-ras Point Mutations**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)/Sex</th>
<th>Diagnosis</th>
<th>Mutation</th>
<th>Karyotype</th>
<th>Position in Fig 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.F.</td>
<td>2.7/f</td>
<td>Early B-precursor ALL</td>
<td>N12p1/G → A/Gly → Ser*</td>
<td>46,XX, del(16)(q23)</td>
<td>a 6</td>
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<tr>
<td>K.L.</td>
<td>6.2/f</td>
<td>C-ALL</td>
<td>N12p1*</td>
<td>46,XX, -8, +mar, i(7(19))</td>
<td>b 13</td>
</tr>
<tr>
<td>H.H.</td>
<td>3.6/f</td>
<td>C-ALL</td>
<td>N12p2</td>
<td>57,XX, +X, +1, +4, +6, +11, +14, +17, +18, +21, +mar, (t1;19)(q23;p13.3)</td>
<td>a 17</td>
</tr>
<tr>
<td>J.P.</td>
<td>7.6/m</td>
<td>Early B-precursor ALL</td>
<td>N13p1/G → T/Gly → Cys</td>
<td>46,XX,(10:11)</td>
<td>a 12</td>
</tr>
<tr>
<td>S.C.</td>
<td>3.9/f</td>
<td>C-ALL</td>
<td>N13p1</td>
<td>54,XX, +X, +4, +6, +15, +17, +17, +18, +mar</td>
<td>a 22</td>
</tr>
<tr>
<td>S.D.</td>
<td>12.3/m</td>
<td>T-ALL</td>
<td>N13p2*</td>
<td>46,XY,inv(14)(q11q32)</td>
<td>b 15</td>
</tr>
</tbody>
</table>

Abbreviations: pos., position; Nctt, Subst., nucleotide substitution; AA Subst., amino acid substitution; CR, complete remission; A, adenine; C, cytosine; T, thymidine; Gly, glycine; Ser, serine; Cys, cysteine.

*Mutation undetectable in CR.

**Table 2. Clinical and Laboratory Characteristics**

<table>
<thead>
<tr>
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<th>No ras Mutations</th>
<th>ras Mutations</th>
<th>P Value*</th>
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<tbody>
<tr>
<td>Age at diagnosis (mean, yr)</td>
<td>6.5</td>
<td>6.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Age &lt;2 yr or &gt;10 yr</td>
<td>27</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>51:43</td>
<td>2:4</td>
<td>.42</td>
</tr>
<tr>
<td>Race (nonwhite)</td>
<td>12</td>
<td>2</td>
<td>.20</td>
</tr>
<tr>
<td>Hepatomegaly†</td>
<td>37</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>Splenomegaly†</td>
<td>43</td>
<td>2</td>
<td>.7</td>
</tr>
<tr>
<td>Leukoocyte count &gt;25 × 10\textsuperscript{9}/L</td>
<td>51</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>Hemoglobin &gt;9 g/dL</td>
<td>43</td>
<td>1</td>
<td>.23</td>
</tr>
<tr>
<td>Platelet count &gt;100 × 10\textsuperscript{12}/L</td>
<td>28</td>
<td>3</td>
<td>.38</td>
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<tr>
<td>FAB classification L1-L2</td>
<td>84:10</td>
<td>4:2</td>
<td>.15</td>
</tr>
<tr>
<td>T-ALL</td>
<td>20</td>
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<tr>
<td>&gt;47 Chromosomes</td>
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<td>1.0</td>
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<tr>
<td>Chromosomal translocations</td>
<td>45</td>
<td>3</td>
<td>1.0</td>
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</table>

*P value by Fisher exact test (two-tailed).
†Extending 5 or more cm below the costal margin.

**Fig 2.** Disappearance of N-ras mutation with complete remission in a patient with early B-precursor ALL. DNA from PBLs from a healthy donor (nl PBL, first lane); T-lymphoblastic MOLT-4 cell line (second lane); leukemic bone marrow obtained from patient S.F. (L25 at diagnosis (third lane) and in remission (fourth lane, sample from bone marrow [Rem. BM]); fifth lane, peripheral blood (Rem. PB) was amplified for N-ras exon 1 as described in Materials and Methods. DNAs were then analyzed for presence of a mutation at the first position of N-ras codon 12, as described in the legend to Fig 1.
RAS GENE MUTATIONS IN CHILDHOOD ALL

Table 3. Differences in Clinical Course Between Patients With Mutations Compared With Those Without ras Mutations

<table>
<thead>
<tr>
<th>Mutations</th>
<th>N</th>
<th>P Value</th>
</tr>
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</table>
| Presence of gene amplification of several oncogenes in malignancies, particularly N-myc and c-erbB2/neu in neuroblastoma and breast cancer, respectively, correlates with a clinical outcome that differs significantly from patients who do not exhibit these alterations. We found no apparent difference in the clinical, demographic, and laboratory data of the patients with ras mutations compared with those without these mutations. However, a significant correlation was observed between presence of N-ras mutations and an adverse outcome. Although this series must be considered preliminary because of the small number of patients with N-ras mutations it suggests that a mutated ras gene represents an independent prognostic variable. It is possible that the biologically more aggressive leukemias (which are more likely to relapse) have developed ras gene mutations. Additional large studies of N-ras gene mutations in childhood ALL are indicated to confirm that these mutations have therapeutic implications.

In recent years, a major focus of molecular cancer research has been the analysis of genes that may be causative in carcinogenesis (oncogenes). Point mutations at distinct regions of exons 1 or 2 of all three ras genes have been detected in a number of human malignancies, including leukemia. More recently, studies examining the occurrence of these mutations were aided by the advent of PCR, which allows fast and efficient screening of large numbers of samples. In this study, we screened a large population of children with ALL. In a modification of slot-staining of amplified products, we electrophoresed the samples on agarose gels and transferred them onto nylon membrane, which allowed an estimate of the efficiency of amplification. Also, it allowed comparison of the molecular weight of the hybridization signal (see below) with the size of the PCR product, thereby facilitating detection of cross-hybridization to aberrantly amplified products of inappropriate size.

We detected a 6% incidence of point mutations at exon 1 of the N-ras gene in childhood ALLs. We found no mutations at either exon 2 of N-ras or exons 1 and 2 of H- and K-ras. This is consistent with and extends a previous study. We did not identify a tendency for a particular nucleotide to be mutated, but found that any of four nucleotide positions of codons 12 or 13 of N-ras were involved.

Presence of gene amplification of several oncogenes in malignancies, particularly N-myc and c-erbB2/neu in neuroblastoma and breast cancer, respectively, correlates with a clinical outcome that differs significantly from patients who do not exhibit these alterations. We found no apparent difference in the clinical, demographic, and laboratory data of the patients with ras mutations compared with those without these mutations. However, a significant correlation was observed between presence of N-ras mutations and an adverse outcome. Although this series must be considered preliminary because of the small number of patients with N-ras mutations it suggests that a mutated ras gene represents an independent prognostic variable. It is possible that the biologically more aggressive leukemias (which are more likely to relapse) have developed ras gene mutations. Additional large studies of N-ras gene mutations in childhood ALL are indicated to confirm that these mutations have therapeutic implications.

By the oligonucleotide hybridization assay and DNA sequencing of PCR product, both the mutated and the normal, wild-type alleles were detected in all cases of ras mutations. This could reflect contamination of the samples with nonleukemic cells, but most likely indicates heterozygosity of the mutation, and therefore suggests dominance of the mutated allele. This observation has also been reported by others.

In the three cases where remission samples of the patients with ras mutations were available, the mutation was undetectable. One patient remains in remission while the other two have relapsed. Although ras gene mutations might serve as a molecular marker for the leukemic clone, the assays used by us in this study are not sensitive enough to allow detection of small quantities of mutated ras gene DNA and would not be useful in the diagnosis of minimal residual disease. A modification of the oligonucleotide hybridization assay offers the potential for detecting ras mutations against a high background of unmutated sequences.

Mutations of the N-ras gene alone are probably insufficient to cause leukemia. Other as yet unknown events are probably necessary to complement the alterations described in this study. Murine studies have suggested that altered nuclear oncogenes such as c-myec can complement mutated ras genes, leading to full transformation of fibroblasts. All of our patients with ras mutations had chromosomal abnormalities, but no one chromosomal abnormality appeared more frequently than others. Very recently, studies have
shown that, in addition to structural changes in ras genes due to point mutations at codons 12, 13, or 61, overexpression of ras may be of importance in transformation. Cohen and Levinson demonstrated that a second mutation in intron 4 of the H-ras oncogene of the human T24 bladder carcinoma cell line (also mutated at codon 12) can cause a 10-fold increase in expression of the mutated allele. One of our patients (H.H.) with an N-ras mutation also had trisomy of chromosome 1, which contains the N-ras gene. Studies of N-ras gene expression in patients with point mutations may provide further insights into the biology of leukemogenesis.

ACKNOWLEDGMENT

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REFERENCES

7. Gibbs JB, Sigal IS, Poe M, Scolnick EM: Intrinsic GTPase
activity distinguishes normal and oncogenic \textit{ras} p21 molecules. Proc Natl Acad Sci USA 81:5704, 1984


