RAS Oncogene Mutations are Rare Late Stage Events in Chronic Myelogenous Leukemia

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DNA from bone marrow and peripheral blood samples of 44 chronic myelogenous leukemia (CML) patients were analyzed for the presence of mutations of codons 12, 13 or 61 of the N-*ras*, H-*ras*, or K-*ras* genes. In seven patients, samples were available from both their chronic phase and blast crisis. A total of 29 samples examined were at chronic phase and 22 were at blast crisis (eight lymphoid, eight myeloid, and six undifferentiated). No mutations were identified in N-*ras* or H-*ras*. Two patients in myeloid blast crisis had K-*ras* mutations, one patient at codon 12, the other at codon 13. In the former patient the mutation was not present and the latter patient was not tested in chronic phase. Our findings indicate *ras* mutations are an infrequent late stage event in CML that occur in myeloid blast crisis.

In 1985 HIRAI STUDIED eight chronic myelogenous leukemia (CML) patients by the NIH/3T3 cell transfection assay and reported one patient who had a mutation of the N-*ras* gene.[1] Subsequently, contradictory results have been reported. Studies of 35 CML patients by Janssen et al[2] and Bos[3] failed to identify point mutations for any of the *ras* genes using polymerase chain reaction (PCR) amplification of *ras* specific sequences and mutation specific synthetic oligonucleotide probes. However, Liu et al, using the same technique, identified *ras* mutations in four of 12 patients studied.[4] Since three of the four mutations were in blast crisis, the implication was made that *ras* mutations were associated with a late stage event leading to the progression of CML from chronic phase to blast crisis.[4]

To define further the incidence of *ras* gene point mutations and their role in the progression of CML from the chronic phase to blast crisis, we investigated the occurrence of point mutations at codons 12, 13, and 61 of the N-*ras*, H-*ras*, and K-*ras* genes by PCR and sequence specific oligonucleotide (SSO) probe hybridization in CML patients treated at the University of Texas M.D. Anderson Cancer Center.

MATERIALS AND METHODS

Patients and DNA isolation. A total of 51 fresh peripheral blood and bone marrow aspirates were obtained from 44 Philadelphia-positive CML patients after they were advised of procedures and attendant risks, and informed consent was obtained in accordance with institutional guidelines. In seven patients samples were obtained in both their chronic phase and blast crisis. Twenty-nine samples were in chronic phase and 22 were in blast crisis (eight lymphoid, eight myeloid, and six undifferentiated). DNA was prepared by standard techniques.[5]

Synthetic oligonucleotides and PCR. Synthetic oligonucleotides of 20 bases in length were obtained for primer amplification and SSO probe panel hybridization specific for codons 12, 13, and 61 of the N-, H-, and K-*ras* genes (*Ras* mutalyzer kit, CLONTECH Laboratories, Inc, Palo Alto, CA).[6] One microliter of DNA was amplified for 40 cycles with denaturation at 95°C for two minutes, annealing at 50°C for two minutes, and extension at 72°C for two minutes. As positive controls cell lines HL-60 with a N-*ras* codon 61 mutation and SW480 with a K-*ras* codon 12 mutation were used.[7,8] The efficiency of amplification was evaluated by electrophoresis and only the reactions resulting in the appropriate size band were either transferred or dot blotted. The SSO panel of wild type and mutated *ras* probes were ^{32}P-end-labeled (specific activity >5 MCi/pmol). Hybridization, washing conditions, and autoradiography were performed as described.[9]

RESULTS

Forty-four patients demonstrated only wild type sequences for N-*ras* with no codon 12, 13, or 61 mutations. The HL-60 control demonstrated both wild type and mutated (codon 61, CTA) sequences, as has been previously described (Fig 1A).[7]

Mutant K-*ras* sequences were identified in two of the 44 patients studied. Both patients were in myeloid blast crisis and demonstrated both wild type and mutant sequences. The first patient had a K-*ras* codon 12 mutation substituting valine (GTT) for glycine (GGT) (Fig 1B). A sample obtained during chronic phase of this patient was normal for K-*ras*, H-*ras*, and N-*ras*. The second patient had a K-*ras* codon 13 mutation substituting aspartic acid (GAC) for glycine (GGC) (Fig 1B). Unfortunately, no chronic phase sample was available in this patient for study. The SW480 control as reported was absent on the wild type screen with only sequences for a K-*ras* codon 12 mutation (GTT) (Fig 1B).[8] The remaining 42 patients were normal for codons 12, 13, and 61 with no evidence of mutations.

No mutations were identified in the 44 patients studied for H-*ras* codon 12, 13, or 61. All of the patients demonstrated only wild type sequences.

DISCUSSION

Recent studies using PCR and SSO probe hybridization to *ras* amplified DNA in acute myeloid leukemias indicate that approximately 20% have mutated *ras* genes.[3,10,11] In solid tumors such as colon and pancreatic carcinoma, the incidence ranges from 40% to 97%.[12-14] The incidence in CML of mutations has ranged from none to 33% of patients studied,

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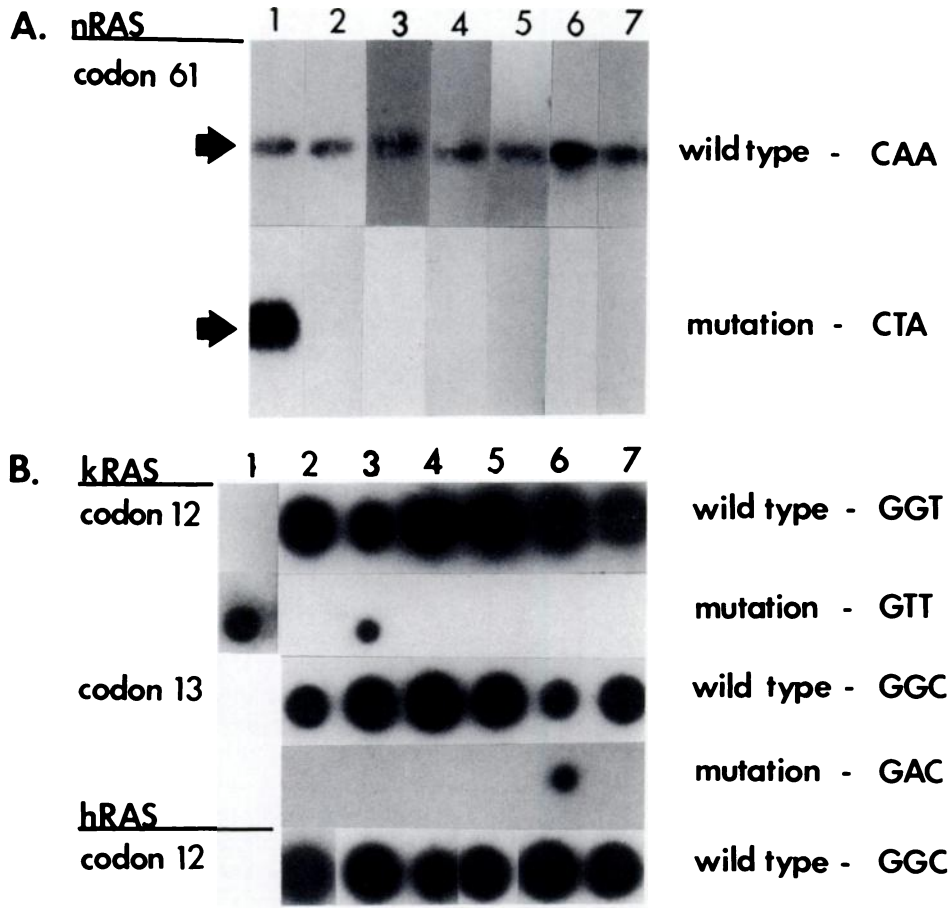

Fig 1. (A) Autoradiograms of identical gels transferred and probed for N-*ras* codon. Top row: arrow indicates the expected size band of 108 base pairs hybridized with the wild type (WT) probe for N-*ras* codon 61, two-hour exposure (lane 1, HL-60; Lane 2, chronic phase sample; lane 3, myeloid blast crisis sample from the patient in lane 2; lanes 4 to 7, additional blast crisis patient samples). Bottom row: point mutation of HL-60 (lane 1) hybridized with the N-*ras* codon 61 leucine (CTA) probe, 15-hour exposure. (B) Autoradiograms of representative dot blots with amplified DNA for K-*ras* and H-*ras*. Top row: hybridized with WT probe for K-*ras* codon 12 demonstrating the absence of normal sequences in SW480 (lane 1) and the presence of WT sequences in the patient samples (lane 2, chronic phase; lane 3, myeloid blast crisis sample from the patient in lane 2; lanes 4 to 7, additional blast crisis samples). Second row: hybridized with mutant probe substituting valine (GTT) for the normal glycine (GGT) at codon 12 and demonstrating the expected mutation in SW480 (lane 1) and the finding of a mutation in a myeloid blast crisis sample (lane 3) that is not present in the chronic phase sample of the patient (lane 2). Third row demonstrates the presence of k-*ras* codon 13 WT type sequences in all of the CML patients shown (lanes 2 to 7). Fourth row: presence of a point mutation containing aspartic acid (GAC) substituted for the normal glycine at K-*ras* codon 13 in a CML myeloid blast crisis patient (lane 6). Fifth row: demonstrates the presence of WT H-*ras* codon 12 sequences (lanes 2 to 7) with no mutations identified in any of the patients studied (not shown).

with most of the mutations found in blast crisis.[2-4] The incidence in our study indicates that it is a relatively infrequent event since only two of the 44 patients (4%) had a *ras* mutation.

Our findings do support that concept that *ras* mutations occur as a late event in CML.[4] Of the six cases with mutations that have been reported in this study and the literature,[4] five have occurred in blast crisis patients and our data suggest that they tend to occur in myeloid blast crisis. Furthermore, our findings of a K-*ras* codon 12 mutation at blast crisis when it was not present during chronic phase suggests that *ras* mutations may be a late event in some CML patients. Unfortunately, no other studies have examined serial samples and the lineage of the other patients reported with mutations is not known.

The finding of *ras* mutations as a late event in CML is different from those in carcinoma and myelodysplasia (MDS). A recent colon carcinoma study by Vogelstein et al suggested that while *ras* mutations may not be part of the first genetic alterations to occur during the development of colorectal tumors, they often were early events in tumorigenesis.[13] These findings are consistent with those in MDS where *ras* mutations appear to be a relatively early event and may serve as a marker for progression to leukemia.[4,15] An understanding of the biologic significance of *ras* mutations and their role in carcinogenesis and disease progression will be aided by delineating the genetic events which produce them.

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