CONCISE REPORT

Relationship Between an Activated N-ras Oncogene and Chromosomal Abnormality During Leukemic Progression From Myelodysplastic Syndrome

By Hisamaru Hirai, Michiko Okada, Hideaki Mizoguchi, Hiroyuki Mano, Yukio Kobayashi, Junji Nishida, and Fumimaro Takaku

The relationship between chromosomal abnormality and oncogene activation was investigated during leukemic progression in two patients with myelodysplastic syndrome (MDS). Both patients had partial or complete deletion of chromosome 5 in metaphase cells obtained throughout the progression to leukemia. Analysis with specific oligonucleotide probes revealed that bone marrow cells containing an activated N-ras oncogene proliferated in a dominant manner during the process of leukemic conversion in both patients. These observations suggest that the chromosomal abnormality may precede activation of the N-ras gene in these patients, and that both the chromosomal abnormality and the activated N-ras oncogene contribute to the development of leukemia.

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Table 1. Clinical Characteristics at the Time of Sampling of Bone Marrow Cells

<table>
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<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>Sample No.</th>
<th>Disease Duration (mo)</th>
<th>Peripheral Blood</th>
<th>Bone Marrow</th>
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</thead>
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<td></td>
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<td>Hb (g/dL)</td>
<td>WBC (x10^9/L)</td>
<td>Platelet (x10^9/L)</td>
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<td>34/M</td>
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<td>30</td>
<td>6.0</td>
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<td></td>
<td>2</td>
<td>40</td>
<td>6.3</td>
<td>1,200</td>
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<td></td>
<td>3</td>
<td>44</td>
<td>6.1</td>
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<tr>
<td>RAEB</td>
<td>54/M</td>
<td>1</td>
<td>11</td>
<td>8.2</td>
<td>2,600</td>
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<tr>
<td></td>
<td></td>
<td>2</td>
<td>17</td>
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<td>1,600</td>
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<td>3</td>
<td>20</td>
<td>5.9</td>
<td>104,600</td>
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</tbody>
</table>

Table 1. Clinical Characteristics at the Time of Sampling of Bone Marrow Cells

Abbreviations: H, hypercellular; N, normocellular; 5q−, del(5)(q15q33); NT, not tested.

MATERIALS AND METHODS

Sources of DNA. Bone marrow cells were collected from two MDS patients: one with refractory anemia (RA) and the other with RA with excess of blasts (RAEB) at first diagnosis. The diagnosis of MDS fulfilled the criteria of the French-American-British (FAB) classification. Clinical characteristics of patients at the time of sampling of marrow cells are shown in Table 1. Normal marrow cells from healthy volunteers were obtained with informed consent and were examined as normal controls.

Synthetic oligonucleotide probes. Synthesis of oligonucleotide probes was carried out as described. The probes N13-1C (5'-GGAGCAGGTCGTTGGAGA-3') and N61-wt (5'-TACTCTTCTTGACGCTG-3') were synthesized using a 20-mer synthetic template and a 8-nucleotide (mer) primer complementary to the 3' end of the 20-mer. This primer-template mixture was incubated with [α-32P]dGTP, [α-32P]dTTP, cold dCTP, and dATP, and DNA polymerase I (Klenow fragment).

Direct gel hybridization. High molecular weight DNAs were extracted fromuffy coat cells of heparinized marrow. Twenty micrograms of DNA were digested with PstI and electrophoresed on 0.5% agarose gels. Gels were denatured in 0.4 mol/L NaOH, 0.8 mol/L NaCl, neutralized in 0.5 mol/L Tris-HCl (pH 7.4), 1.5 mol/L NaCl, and dried. Dried gels were then hybridized at 53°C with the N13-IC probe or at 50°C with the N61-wt probe in 5 x SSPE (1 x SSPE is 10 mmol/L sodium phosphate (pH 7.0), 0.18 mol/L NaCl, 1 mmol/L EDTA), 0.3% SDS and 10 μg/mL sonicated salmon sperm DNA. Hybridized gels were washed in 2 x...
N-ras ACTIVATION AND CHROMOSOMAL ABNORMALITY

Fig 1. Hybridization of synthetic oligonucleotide probes to DNAs of bone marrow cells from two patients with RA or RAEB. Sample numbers are identical to those in Table 1. Normal human DNA (N) was used as a control.

SSPE, 0.1% SDS at room temperature, in 5 x SSPE, 0.1% SDS at 53°C for 15 minutes, and finally in the same solution at 63°C for the N13-1C probe or at 59°C for the N61-wt probe for five minutes. Gel membranes were autoradiographed for three to five days using intensifying screens. Hybridized mutated N-ras bands were quantitated by scanning the autoradiogram with a densitometer.

Cyto genetic studies. Chromosome analysis was performed on synchronized or unsynchronized cultures from each bone marrow sample using a Q-banding technique. The synchronization method with methotrexate for a routine air-drying technique has been described.16

RESULTS

The N-ras gene of both cases presented here was found to contain a point mutation from G to C at the first letter of codon 13, using an in vivo selection assay in nude mice with transfected NIH3T3 cells as described.14 In the present studies, the point mutation was detected in bone marrow cell DNA by hybridization with oligonucleotide N13-1C. This N13-1C probe encompasses the sequence around mutant codon 13, and hybridized only to mutant DNA under high stringency conditions. Bone marrow cell DNA was prepared from both patients at different stages of leukemic progression, and probed with N13-1C (Fig 1). In both cases, the N13-1C probe failed to give a clear hybridizing signal in the first sample, but the strength of the signal increased significantly in samples 2 and 3. The data were quantitated by densitometry, and the results are plotted in Fig 2. These results suggest that the intensity of the hybridizing signal is proportional to the percentage of blasts in each case, since the internal control N61-wt probe, corresponding to the normal sequence of the N-ras gene surrounding codon 61, hybridized equally well to DNA from all samples. These observations indicate that proliferative blast cells contain the point mutation at codon 13 of the N-ras oncogene.

DISCUSSION

Partial or complete loss of chromosome 5 has been observed in patients with RA or with acute nonlymphocytic leukemia arising either de novo or secondary to cytotoxic therapy for a previous malignant disease.13,17-24 The variability of the breakpoints noted in the deletion of chromosome 5 suggests that the event underlying malignant transformation is the loss of a critical DNA sequence rather than the consistent juxtaposition of two genes (as in the case of c-abl and bcr in chronic myelogenous leukemia.25). Partial loss of chromosome 5 is considered to be necessary but not sufficient for malignant transformation, although patients with a single 5q- tend to have a mild course and cases with a combined deletion 5 have a relatively poor prognosis.12,13,24 Thus, an additional event is considered to have a role in transformation to acute nonlymphocytic leukemia.

In our two cases, the deletion of part or all of chromosome 5 has been observed in metaphase cells from early stages of the disease, indicating that the chromosomal deletion may provide a growth advantage to the bone marrow cells. Our studies with transfection assay14 and oligonucleotide hybridization (Figs 1 and 2) suggest that the second event in leukemic progression is the activation of the N-ras gene in one or a small number of cells. These results indicate that the activated N-ras oncogene is contributing in a dominant manner to the process of leukemic progression from MDS. Thus, developed leukemic cells were found to contain both the abnormality of chromosome 5 and the point mutation of an N-ras gene. It seems likely that activation of the N-ras oncogene probably occurs after initiation of leukemogenesis. These observations demonstrate potential relationships between chromosomal abnormality and oncogene activation, and provide insight into the nature of leukemogenesis in other leukemia cases with chromosomal abnormalities.

REFERENCES

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