Localization of the G-CSF Gene on Chromosome 17 Proximal to the Breakpoint in the t(15;17) in Acute Promyelocytic Leukemia

By Ruth N. Simmers, Lorna M. Webber, M. Frances Shannon, O. Margaret Garson, Gordon Wong, Mathew A. Vadas, and Grant R. Sutherland

The human granulocyte-colony stimulating factor gene (G-CSF) is localized at 17q11.2–q21, the region of one of the breakpoints in the 15:17 chromosome translocation specific for acute promyelocytic leukemia (APL). As G-CSF induces differentiation and loss of tumorigenicity in myeloid leukemic cells or cell lines, it was possible that the translocation in APL involved the DNA of the G-CSF coding region or its regulatory region. In situ hybridization to myeloid leukemic cells or cell lines, it was possible that the translocation using a G-CSF cDNA clone revealed that the coding region of this gene is proximal to the t(15;17) breakpoint on chromosome 17. Southern analysis of DNA from patients with the APL translocation showed no differences in hybridization between normal and leukemic cells. These results indicate that the G-CSF coding sequence is not disrupted by the chromosomal rearrangement characteristic of APL.

The specific chromosome rearrangement observed in the malignant cells of patients with acute promyelocytic leukemia (APL) involves chromosomes 15 and 17, but breakpoints in the translocation remain controversial, with that on chromosome 17 being localized variously from 17q11.2 to 17q22.1,4 The gene that encodes for human granulocyte-colony stimulating factor (G-CSF) has been mapped to the region 17q11.2-21 by in situ hybridization.5 G-CSF normally stimulates progenitor cells to proliferate and differentiate into neutrophils,6,7 and will also stimulate leukemic cells or cell lines to differentiate into mature neutrophils.8,11 It is therefore possible that the 15;17 translocation in APL involves either the G-CSF gene or its regulatory region.

As the specific chromosome rearrangement in APL divides 17q into two regions, it is of interest to know to which of these regions G-CSF maps and whether the translocation alters the in situ hybridization patterns. We have studied the t(15;17) in two patients with APL using in situ hybridization with the G-CSF probe and in four patients using Southern analysis.

MATERIALS AND METHODS

DNA studies. Bone marrow cells from patients with APL were cultured for 24 hours before being synchronized with 0.1 μmol/L fluorodeoxyuridine.12 The block was released by the addition of 20 μmol/L bromodeoxyuridine and the chromosomes harvested 8½ hours later after 30 minutes’ exposure to colcemid. These chromosomes were banded and photographed prior to in situ hybridization.

This was carried out using a cDNA clone consisting of the entire coding region of the G-CSF gene in the plasmid pXMT2, to these bone marrow and to normal male lymphocyte chromosomes as described by Simmers et al.13 Kodak NTB-2 (Rochester, NY) and Ilford L4 (Essex, UK) nuclear research emulsions were used for autoradiography.

Southern analysis was performed, using the G-CSF probe, to DNA from normal and leukemic cells of four patients with APL and the t(15;17). (DNA was kindly supplied by Professor A. Morley and Dr L. Ashman.) The DNA was cut by four restriction enzymes: Eco RI, Bam HI, Bgl II, and Hind III.

Patients. Two patients with APL were studied using in situ hybridization. Patient ML was a 72-year-old woman who presented with APL (M3) in June 1986. She died from uncontrollable bleeding shortly after admission. The leukemic cell karyotype showed the typical 46,XX,t(15;17)(q22;q21) in 95% of metaphases examined. Patient SD was a 15-year-old boy who presented with APL in November 1984. The leukemic karyotype was 46,XY,t(15;17)(q22;q21),i(17q-): an isochromosome of the partial deletion 17q is a recognized variant of the t(15;17) in APL.14 The patient achieved remission, but subsequently relapsed on several occasions and although additional chromosome abnormalities were found at relapse, the t(15;17),i(17q-) was always present, with approximately 20% of metaphases having an additional i(17q-). The patient died 21 months after diagnosis.

RESULTS

Examination of 25 normal metaphases probed for G-CSF revealed significant label on the long arm of chromosome 17: 22 out of 108 silver grains (20%) were seen over this region (P < 0.05 using cumulative Poisson probabilities), indicating that the probe had hybridized to G-CSF on 17q. Chromosomes from 121 metaphases of patient ML had 16 silver grains (6.4% of a total of 249 silver grains over all chromosomes; P < 0.05) over the region 17cen to 17pter on the normal chromosome 17; 22 (8.8% of 249; P < 0.05) over 17cen to 15pter on the derived chromosome 17; and four (1.6% of 249;NS) over both 15q15 to 15pter and 15q15 to 17qter on the normal and derived chromosomes 15, respectively. Chromosomes from 130 metaphases of patient SD had 14 silver grains (4.9% of a total of 287 silver grains over all chromosomes; P < 0.05) over 17cen to 17pter on the normal chromosome 17; 44 (15.3% of 287; P < 0.05) over the iso17q; and four (1.4% of 287;NS) and five (1.7% of 287;NS) over 15q15 to 15pter and 15q15 to 17qter on the normal and derived chromosomes 15, respectively. In sum-
DISCUSSION

The chromosome 17 breakpoint of the translocation in APL cells has been located variously from 17q11.2 to 17q22. This region overlaps with the localization of G-CSF at 17q11.2-21 recently described by Simmers et al. Thus, while the location of the G-CSF gene has not been defined more precisely by the present results, the position of the coding region with respect to the breakpoint and its probable noninvolvement in the translocation have been revealed.

The oncogene c-erbA has also been mapped proximal to the chromosome 17 breakpoint in APL and no rearrangement of the c-erbA gene resulting from this translocation has been detected. In a case of undifferentiated acute leukemia found to have a t(17;21)(q21;q22;q22), the c-erbA gene was localized proximal to this breakpoint at 17q21-22. In addition, the nerve growth factor receptor gene (NGFR) has been mapped distal to this breakpoint at 17q22. Although cytogenetically similar, the point of translocation in this undifferentiated leukemia is not necessarily the same as the DNA level as in t(15;17) in APL, so it cannot be assumed that NGFR is actually distal to the breakpoint in APL. No rearrangement of the NGFR gene was detected in chromosomes with the t(17;21) or the APL translocation.

As the G-CSF probe used for in situ hybridization in our studies contains the entire coding region of the G-CSF gene, the results indicate that these coding regions are proximal to the site of rearrangement in APL. However, this information does not exclude the possibility of the rearrangement occurring through a region of DNA flanking the coding sequence, affecting the regulation of G-CSF production.

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