RAPID COMMUNICATION

Cytogenetic and Molecular Delineation of a Region of Chromosome 7 Commonly Deleted in Malignant Myeloid Diseases

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Loss of a whole chromosome 7 or a deletion of the long arm, del(7q), are recurring abnormalities in malignant myeloid diseases. To determine the location of genes on 7q that are likely to play a role in leukemogenesis, we examined the deleted chromosome 7 homologues in a series of 81 patients with therapy-related or de novo myelodysplastic syndrome or acute myeloid leukemia. Our analysis showed that the deletions were interstitial and that there were two distinct deleted segments of 7q. The majority of patients (65 of 81 [80%]) had proximal breakpoints in bands q11-22 and distal breakpoints in q31-36; the smallest overlapping deleted segment was within q22. The remaining 16 patients had deletions involving the distal q arm with a commonly deleted segment of q32-33. To define the proximal deleted segment at q22 at a molecular level, we used fluorescence in situ hybridization with a panel of mapped yeast artificial chromosome (YAC) clones from 7q to examine 15 patients with deletion breakpoints in q22. We determined that the smallest overlapping deleted segment is contained in a well-defined YAC contig that spans 2 to 3 Mb. These studies delineate the region of 7q that must be searched to isolate a putative myeloid leukemia suppressor gene, and provide the necessary cloned DNA for more detailed physical mapping and gene isolation.

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RECURRING CHROMOSOMAL abnormalities are characteristic of human malignant diseases, particularly the leukemias and lymphomas.1-3 To date, the major emphasis of the molecular analysis of the chromosomal abnormalities in hematologic malignant diseases has involved the recurring translocations, in which two genes are juxtaposed, resulting in the activation of an oncogene in a dominant fashion.4 However, the loss of genetic material in human tumors has received considerable attention, particularly in solid tumors. This genetic change may contribute to the development of human neoplasms by inactivating both alleles of tumor suppressor genes.5-7 The observation of recurring cytogenetic deletions in malignant cells has provided a starting point for the positional cloning of a number of tumor suppressor genes that are important in the development of solid tumors.

The identification of recurring chromosomal deletions in the hematologic malignant diseases suggests that, similar to that of solid tumors, the inactivation of tumor suppressor genes may be involved in the pathogenesis of some leukemias.8 In fact, genetic and biochemical studies of leukemia cells from children with neurofibromatosis I (NF1), and of hematopoietic cells from strains of mice with a targeted disruption of murine NFI, have shown that NFI functions as a tumor suppressor gene in immature myeloid cells by negatively regulating the p21 signaling pathway.9-12 With respect to the myeloid disorders, recurring chromosomal deletions include the del(5q), del(7q), del(9q), del(11q), del(12p), del(13q), and del(20q).1 A commonly deleted segment has been identified by physical mapping with DNA probes for several of these rearrangements [del(5q), del(11q), del(12p), del(13q), and del(20q)].1,3-6 These cytogenetic findings implicate a number of discrete regions of the genome that are likely to contain other tumor suppressor genes that are inactivated during myeloid leukemogenesis.

Loss of a whole chromosome 7 (−7) or a deletion of the long arm of this chromosome [del(7q)] are common recurring abnormalities in malignant myeloid disorders, and are observed in three general contexts.17 First, −7/del(7q) is noted in the malignant cells of 10% of patients with a myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) arising de novo.2,4 Second, abnormalities of chromosome 7 are observed at the highest frequency in MDS or AML arising after cytotoxic therapy for another disorder, usually a primary malignancy (therapy-related or t-MDS/t-AML), or after occupational or environmental exposure to mutagens.10-12 In our recently updated series of 246 consecutive patients with t-MDS/t-AML, 230 (94%) had a clonal chromosomal abnormality (Le Beau et al13 and Le Beau et al, unpublished results), and 175 patients (71%) had a clonal abnormality leading to loss or deletion of chromosome 5 and/or 7. Among these 175 patients, 87 had a loss of chromosome 7, 18 had a del(7q), 21 had loss of 7q as a result of an unbalanced translocation, and 1 patient had a balanced translocation involving 7q22. Fifty-seven patients had abnormalities of both chromosomes 5 and 7. Overall, 105 patients (42%) had abnormalities of chromosome 5, and 127 (52%) had abnormalities of chromosome 7.

Third, leukemias arising in individuals with constitutional disorders associated with a predisposition to myeloid leukemias, eg, Fanconi anemia, congenital neutropenia, and NFI, are frequently characterized by −7/del(7q), often as the sole cytogenetic abnormality.14 Taken together, these data are consistent with the hypothesis that inactivation of a tumor

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suppressor gene located on 7q is a common event that contributes to the pathogenesis of malignant myeloid disorders in a number of biological and clinical contexts.

We and others have proposed that the long arm of chromosome 7 contains a tumor suppressor gene and that this gene is likely to be located within a commonly deleted segment in patients who have a del(7q). By cytogenetic analysis of 81 patients with a del(7q), we have identified two commonly deleted segments, suggesting that 7q may contain more than one myeloid leukemia-related gene. In addition, we have used fluorescence in situ hybridization (FISH) to delineate a commonly deleted segment at 7q22 using molecular probes corresponding to a well-defined yeast artificial chromosome (YAC) contig mapping to this region of the genome.

MATERIALS AND METHODS

Patients. We examined bone marrow (BM) or peripheral blood (PB) specimens from 81 patients who were diagnosed and treated at the University of Chicago Medical Center or referred to our cytogenetics laboratory between 1970 and 1996 from other metropolitan Chicago hospitals. The diagnosis and subclassification of MDS or AML was based on morphological and cytochemical studies of PB smears, BM aspirates, and biopsy specimens obtained before therapy, according to the FAB Cooperative Group criteria. Cytogenetic analysis was performed with quinacrine fluorescence and trypsin-Giemsa banding techniques on BM cells from aspirate or biopsy specimens or on PB cells obtained at the time of diagnosis. We examined metaphase cells from direct preparations or from short-term (24 to 72 hours) unstimulated cultures. Although three cytogeneticists evaluated the deleted homologues in each case, the designation of breakpoints in malignant cells can be somewhat imprecise, particularly when the chromosomes are condensed. Of the 81 patients examined, 8 patients were ascertainment before 1982, when Q-banding was in use, and the average number of chromosome bands observed per haploid cell was 350 to 400. The remaining patients were studied using G-banding methods; during this period, the average length of the chromosomes had improved to >450 bands per haploid cell. Chromosomal abnormalities are described according to the International System for Human Cytogenetic Nomenclature (1995).22

DNA probes. YAC clones used for FISH studies were selected from a physical map of chromosome 7 constructed by sequence-tagged site (STS) content mapping (Green et al23 and Green et al, manuscript in preparation). Yeast cells containing YACs were grown in acid hydrolyzed casein (AHC) media (inoculated with a single colony from an AHC plate), and DNA was prepared using standard procedures.24 DNA was resuspended at a concentration of ~100 ng/μL, and Inter-Alu polymerase chain reaction was performed using the procedures described by Lengauer et al25 and Liu et al.26 The polymerase chain reaction products were biotin-labeled by nick-translation using Bio-11-deoxyuridine triphosphate (dUTP; Enzo Diagnostics, New York, NY).27

FISH. Human metaphase cells were prepared from phytohemagglutinin-stimulated PB lymphocytes or from BM or PB cells from patients with MDS or AML. FISH was performed as described previously.27 Hybridization of biotin-labeled probes was detected with fluorescein-conjugated avidin (Vector Laboratories, Burlingame, CA), and chromosomes were identified by staining with 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI). In some instances, a centromere-specific probe for chromosome 7 (CEP7; Spectrum Orange; Vyssis Inc, Downers Grove, IL) was cohybridized with the YAC probes, to facilitate the identification of the chromosome 7 homologs. Cytogenetic map locations were determined by analyzing the signal relative to the Alu-R banding pattern observed on hybridized chromosomes and the DAPI staining pattern observed using a DAPI-fluorescein isothiocyanate dual-pass filter (Chromatechnology, Brattleboro, VT). For the analysis of leukemia samples, 10 to 15 metaphase cells with an abnormality of 7q were scored by each of two independent observers; in a few instances, only 5 to 9 metaphase cells were available for analysis.

RESULTS

Cytogenetic delineation of the commonly deleted segments. To determine the location of genes on 7q that may be involved in myeloid leukemogenesis, we first examined the breakpoints of the deletions in a consecutive series of 81 patients. This analysis included 26 patients with t-MDS/t-AML and 55 patients who had primary MDS or AML de novo (Fig 1). Our analysis suggested that the deletion breakpoints were heterogeneous and that the deletions were interstitial. In contrast to other recurring deletions in myeloid disorders, eg, del(5q), there may be two distinct deleted segments of chromosome 7. The majority of patients (65 of 81 [80%]) had proximal breakpoints in q11 (25 patients) or q22 (27 patients); the remaining 13 patients in this group had a proximal breakpoint in 7q21. Thirty-five patients had distal breakpoints in q36; less often, distal breakpoints were observed in q22 (10 patients) and q34 (10 patients); bands q31, q32, q33, or q35 were involved in the remaining 10 patients. The identification of patients who had a proximal breakpoint in 7q22 (27 patients) and of other patients who had a distal breakpoint in this band (10 patients) allowed us to refine this commonly deleted segment of 7q to band q22. We have also identified 9 patients with myeloid leukemias who had balanced translocations involving 7q22, providing further support that a commonly deleted segment of 7q may be localized to this chromosomal band (Le Beau et al, unpublished results).

The remaining 16 patients had interstitial deletions involving the distal long arm of chromosome 7. The proximal breakpoints were in q31 (5 patients) or q32 (11 patients). The majority of patients had a distal breakpoint in q36 (10 patients), but distal breakpoints were also observed in q33 (3 patients), q34 (1 patient), and q35 (2 patients). The commonly deleted segment for this group of patients consisted of 7q32-33.

Molecular delineation of the commonly deleted segment at 7q22. To delineate the commonly deleted segment at 7q22 at the molecular level, we performed FISH of 13 YACs distributed along 7q, including 7 YACs within 7q22, to metaphase cells with a del(7q). Because of the limited amount of material available for some patients, we were unable to hybridize each probe to metaphase cells from all of the patients. The YACs were hybridized to leukemia cells from 15 patients who had either a proximal breakpoint (6 patients) or a distal breakpoint (9 patients) within 7q22. The majority of patients had a myeloid neoplasm (MDS/AML de novo, 5 patients; t-MDS/t-AML, 5 patients; chronic myelogenous leukemia in myeloid blast crisis, 1 patient); however, we also examined 4 patients with lymphoid diseases characterized by a del(7q) (acute lymphoblastic leukemia, 2 patients; non-Hodgkin’s lymphoma, 1 patient; and chronic lymphocytic leukemia, 1 patient).
leukemia, 1 patient), who were not included in our cytogenetic analysis of the deletions described above. The genomic origin of each YAC clone on 7q was confirmed by hybridization to normal metaphase cells.23 These studies showed that both the proximal and distal breakpoints were heterogeneous at the molecular level. YAC yWSS1668 and all clones centromeric of this YAC were proximal to the commonly deleted segment and were not deleted in all patients, whereas YAC yWSS3710 and all clones telomeric to this YAC were distal to the commonly deleted segment and were not deleted in all patients (Figs 2 and 3). In all cases, hybridization of the YACs was observed on the normal chromosome 7, suggesting that submicroscopic deletions had not occurred on the normal homolog.

**Cloned coverage of commonly deleted region in YACs.**

The FISH analysis described above indicated that the commonly deleted region of 7q22 was located between YACs yWSS1668 and yWSS3710. As part of a global effort to assemble a fully integrated physical map of human chromosome 7,23,28-30 these two YACs have been mapped to a well-defined, highly redundant YAC contig. The most relevant portion of this contig is shown schematically in Fig 4. The interval defined by the flanking clones yWSS1668 and yWSS3710 (Fig 4) is roughly 2 to 3 Mb in size (E.D. Green, unpublished data). Importantly, this region is fully contained within a set of overlapping clones, with the overlap relationships established by STS content mapping. Among the STSs mapping to this interval are a number of established genetic markers (D7S1503 [sWSS3517], D7S2509 [sWSS1098], D7S2504 [sWSS2506], D7S818 [sWSS2539], D7S796 [sWSS1636], D7S658 [sWSS1097], D7S2446 [sWSS2492], D7S2494 [sWSS3045], D7S1530 [sWSS3516], D7S2545 [sWSS1763], and D7S1841 [sWSS3256]) and one known gene (PSMC2 or MSSI [sWSS1845]; see GenBank D110941). Of note, relevant genes known to reside nearby on 7q, such as the genes encoding erythropoietin (EPO) and plasminogen activator inhibitor 1 (PLANH1) map to other regions on chromosome 7 and not to the contig shown in Fig 4. Thus, the commonly deleted region of 7q22 is fully accessible in cloned form, with the corresponding YACs and STSs representing valuable starting reagents for pursuing the isolation of the myeloid leukemia tumor suppressor gene.

**DISCUSSION**

Defining a commonly deleted segment on 7q in myeloid leukemias is of particular interest because alterations of this chromosome are common, and they arise in a variety of clinical contexts. Our results from cytogenetic and molecular mapping of the deletions of chromosome 7 in malignant myeloid disorders suggest that there are two distinct regions...
Fig 3. FISH of YAC clones to leukemia cells with a del(7q). (A) Biotin-labeled YAC yWSS1668 DNA was hybridized to metaphase cells from patient no. 11 with a del(7)(q22q34). Hybridization signal was detected on both the normal (arrow) and deleted (arrowhead) homologs and, thus, defines the proximal boundary of the commonly deleted segment. (B) Hybridization signal for biotin-labeled YAC yWSS3710 DNA (detected with fluorescein isothiocyanate-avidin) was observed on the normal homolog (arrow) and on the del(7)(q11q22) in patient no. 4, defining the distal boundary of the commonly deleted segment (arrowhead).

of 7q, 7q22 and 7q32-33, that are likely to contain genes involved in the pathogenesis of these disorders. Furthermore, these results suggest that genes located within the same cytogenetic intervals are involved in the pathogenesis of both de novo and therapy-related myeloid diseases. Band 7q22 also appears to be involved in patients who developed myeloid disorders in the context of a constitutional predisposition (Fanconi anemia or NFI), although the cytogenetic data are limited. We have used FISH analysis of malignant cells characterized by a del(7q) to confirm that the deletions are interstitial. Moreover, we have delineated the centromeric commonly deleted segment at 7q22. This region is flanked by YAC yWSS1668 on the proximal side and yWSS3710 on the distal side, with the complete interval covered by a well-defined YAC contig.

The delineation of a commonly deleted segment on 7q has been more difficult than that for deletions of other chromosome, such as 5q. This is largely because loss of a whole chromosome 7 represents the most common abnormality, and relatively few patients with a del(7q) have been available.

Fig 4. YAC contig containing the commonly deleted region of 7q22. A highly redundant YAC-based STS-content map of the region of chromosome 7q22 commonly deleted in myeloid leukemia has been constructed (E.D. Green, manuscript in preparation). For simplicity, the minimal set of YACs from this contig that provide cloned coverage across the interval flanked by clones yWSS1668 and yWSS3710 is depicted schematically. The STSs (named with the prefix “sWSS” followed by a unique number) mapping to these YAC clones are listed along the top, whereas the YACs (named with the prefix “yWSS” followed by a unique number) are shown as horizontal bars. The measured size of each YAC (in kilobases) is indicated below its name. The presence of an STS in a YAC is indicated by a darkened circle at the appropriate position. All information about the STSs is available in Genbank and the Genome Data Base, whereas information about the YACs is available in the Genome Data Base. The established order of STSs from the centromeric (leftward) to 7q telomeric (rightward) ends is indicated. Note that the limited set of YACs shown in this figure do not provide sufficient mapping information to deduce the indicated order of STSs; however, this order is well supported by the complete data set.
for analysis. Kere et al\textsuperscript{12} used restriction fragment-length polymorphisms and pulsed-field gel electrophoresis to localize the proximal breakpoints on 7q in leukemia cells from four adults with MDS or AML and a del(7q). They observed loss of heterozygosity for cDNA probes immediately telomeric of the \textit{EPO} gene at 7q21.3-22, including the \textit{PLANH1} gene, which is located \textasciitilde3 centimorgans (cM) distal to \textit{EPO}. Although cytogenetic analysis of their patients suggested that the entire distal q arm was deleted, analysis with DNA markers showed that the deletions were interstitial.\textsuperscript{33} Lewis et al\textsuperscript{34} examined five patients with MDS and a del(7q) and found that the deletions were interstitial in each case; constitutional heterozygosity of the \textit{TCRB} locus at 7q34 was retained in three patients. By using FISH analysis, Fischer et al\textsuperscript{35} defined a 3- to 4-Mb commonly deleted segment that includes the \textit{PLANH1} and \textit{CUTL1} genes in 7q22, a region that may be close to the commonly deleted segment identified in our studies. Tosi et al\textsuperscript{36} examined a myeloid leukemia cell line with a del(7q) and determined that the breakpoint at 7q22 was proximal to \textit{GNB2}, which is centromeric of \textit{EPO} and \textit{PLANH1}.

Although the studies described above suggest that the commonly deleted segment in 7q22 is close to or contains the \textit{PLANH1} gene, several groups of investigators have identified other regions of 7q22 or even 7q31 as potential locations for a myeloid tumor suppressor locus. For example, Kiuru-Kuhlefelet et al\textsuperscript{37} used microsatellite markers to examine leukemia cells with a del(7q): the allele loss observed in four patients delineated a segment between markers D7S515 and D7S685 that was lost. This region overlaps slightly with the distal end of our commonly deleted segment but extends into q31. Other studies of a few patients with \textendash 7 and unidentified marker chromosomes suggested that loss of a small segment including D7S522 (7q31) may be a critical event in the pathogenesis of some myeloid disorders.\textsuperscript{38}

Johnson et al\textsuperscript{39} recently reported on a family in which multiple members in a 3-generation pedigree had a constitutional inversion of 7q, inv(7)(q22.1;q34). One individual developed MDS, and another had BM hypoplasia; both had the inv(7q). The breakpoint at 7q22.1 was mapped to a YAC containing the asparagine synthetase gene (\textit{ASNS}). Although these results are intriguing, their significance is unclear because the incidence of myeloid disorders is low in family members with the inversion and because the \textit{ASNS} gene has been mapped proximal to \textit{EPO} and \textit{COLIA2}, in a region that retains heterozygosity in many patients with MDS/AML and a del(7q).\textsuperscript{32,33} Moreover, this region is proximal to the commonly deleted segment that we have defined in this study. It is possible that there are multiple genes in 7q21.3-22 that are involved in the pathogenesis of malignant myeloid disorders or that the myeloid diseases in this family are unrelated to the constitutional inversion.

The commonly deleted region of 7q22 defined in our studies is contained in a well-defined contig; the corresponding YAC clones and STSs represent valuable reagents for the isolation of a myeloid leukemia tumor suppressor gene.

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Cytogenetic and molecular delineation of a region of chromosome 7 commonly deleted in malignant myeloid diseases

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