Extensive Cross-Homology Between the Long and the Short Arm of Chromosome 16 May Explain Leukemic Inversions and Translocations


Specific rearrangements of chromosome 16 are well known in acute nonlymphocytic leukemia with abnormal eosinophils. While mapping cosmids relative to breakpoints in chromosome 16 in leukemic cells with fluorescence in situ hybridization (FISH), we have identified three areas of extensive cross-homology between 16p and 16q. Three cosmids among 99 tested showed two large signals on the short arm and one signal on the long arm of chromosome 16. A fourth cosmid showed mainly two signals on the short arm. With the 16p-specific cosmid we can demonstrate that the breakpoints of a pericentric inversion and a reciprocal (16;16) translocation, both of which are characteristic for acute leukemia, map to the most distal of two blocks on the short arm. We suggest that there may be at least two distinct repetitive elements specific for chromosome 16 interdigitated on 16p. The presence of a similar repeat in the short, as well as the long arm of the chromosome, may play a role in the origin of chromosome 16 rearrangements in acute leukemia.

Cancer is now known to be caused by changes in genes. These changes can be brought about by chromosomal rearrangements, such as translocations, deletions, and inversions. A large number of chromosomal rearrangements specific for certain types of malignancy have been delineated.

In 1983, Arthur and Bloomfield\(^2\) and LeBeau et al\(^3\) reported on specific changes of chromosome 16, ie, del(16)(q22) and inv(16)(p13q22) in acute myelomonocytic leukemia (AMML or M4 in the French-American-British [FAB] classification), which is characterized by the presence of bone marrow eosinophils with abnormal granulation, often referred to as M4-eo. In 1984, Testa et al\(^4\) described t(16;16) in a patient with a similar type of leukemia. The inv(16) is a subtle alteration that is difficult to detect with certainty on contracted chromosomes with inferior banding quality, which is a common feature of metaphase chromosomes from leukemic cells. We have designed a sensitive method for rapid detection of inv(16) using two strategically chosen cosmids probes and fluorescence in situ hybridization\(^6\) (FISH). We have also established that the breakpoint in the short arm of chromosome 16 of the inv(16) and the t(16;16) map to the same subregion of 16p.\(^7\) The breakpoint of another translocation involving the short arm of chromosome 16, the t(8;16), associated with acute monoblastic leukemia (AMoL or M5 in the FAB classification) with prominent crythrophagocytosis and thrombophagocytosis,\(^8\) although cytogenetically indistinguishable from the breakpoint of inv(16) or t(16;16), was mapped to a distinct subregion of 16p.\(^12\) Using radioactive in situ hybridization techniques, LeBeau et al\(^11\) reported the splitting of the metallothionein gene cluster (MT) mapped on 16q by the inversion breakpoint. Since this cluster of genes had been cloned, the isolation of the breakpoint seemed a straightforward procedure. However, later studies by Sutherland et al\(^14\) also using in situ hybridization techniques on high resolution banding chromosomes, indicated that the MT gene cluster actually appears to map at 16q13, in fact proximal to the breakpoint. We have investigated the 16p breakpoint of the inversion, because a detailed map of this chromosomal region was already available.\(^15\) The serial mapping of cosmids on chromosome 16 showed extensive cross-homology between the p and q arms of this chromosome.

Fig 1. Schematic representation of the results obtained after hybridization of 99 cosmids to a number of cell lines with breakpoints in the 16p13 region. Hybrid CV 19 contains the der(16) of a t(13;16)(q12.1;p13.1).PK75.4, 46,XY,t(16;16)(q12;p13) (kindly provided by Dr D.M. Carr, Drew Medical School, Los Angeles, CA) is a fibroblast cell line. Loucy is a 45,X,5q-,t(16;20)(p12;q13) cell line from a patient with T-cell acute lymphoblastic leukemia.\(^16\) Cell line PK31.2, 46,XY,t(7;16)(p32;p13) is from the father of a patient with partial trisomy 16p.\(^16\) C55, C60, C129, and C36 are cosmids that map to the region of interest. C36 shows double signals on prometaphase chromosomes, and may recognize a duplication; the breakpoint of PK31.2 separates the two signals (data not shown).

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Fig 2. In situ hybridization of chromosome 16 cosmids on metaphase spreads. (A) Hybridization of cosmid 163 to normal metaphase chromosomes, showing bright signals on 16q22 and two very broad blocks of signals on 16p. (B) Hybridization of cosmid 177 to normal metaphase chromosomes, showing weak signals on 16q, two very broad blocks on 16p, and signals on 18p (arrowhead). (C and D) Hybridization of cosmid 177 to metaphase chromosomes of ANLL M4-EO patients with (C) the pericentric inversion inv(16)(q22p13) showing signals of the distal block of 16p, transported to the q arm of the same chromosome (arrow), and (D) the translocation t(16;16)(q22;p13) showing signals of the short arm of one chromosome 16 transported to the long arm of the other 16 (arrow pointing at der(16)(16qter-16q22::16p13-16pter)). (E) Shows the signals on 16q (arrow). (F) Shows the two blocks on the der(16)(16qter-16p13::16q22-16qter) (arrowhead). (Differentiation between the p-arm and q-arm was done on basis of the DAPI banding pattern.)

MATERIALS AND METHODS

Patients. Bone marrow samples from patients were obtained from the diagnostic service of the cytogenetic laboratories. In addition, samples stored in liquid nitrogen were studied. Histological and immunological analysis of the same samples was performed in parallel. In total, we have investigated bone marrow from eight patients with inv(16) and one with t(16;16).

Cosmids. Chromosome 16 cosmids were obtained from a library prepared from a mouse hybrid cell line containing chromosome 16 as the only human chromosome.16

In situ hybridization. Metaphase spreads were prepared according to standard protocols.6,13 Probes were labeled by nick translation18 in either the presence of biotin-11-dUTP or digoxigenin-11-dUTP and further purified and precipitated as described elsewhere.13 Hybridization and detection of the hybrids was performed as described by Pinkel et al19 and Kievits et al.19 The slides were mounted in antifade medium containing propidium iodide (0.5 μg/mL) and DAPI (0.3 μg/mL) for counterstaining of the chromosomes according to Kievits et al.19 Analysis was performed with a Leitz Aristoplan microscope equipped for fluorescence.
microscopy, and suitable metaphases were photographed on 3M 640 ASA film.

Two-color in situ hybridization. For the detection of a biotinylated probe in one color and the digoxigenated probe in a second color, the following procedure was used: After hybridization, the slides were washed three times for 5 minutes in 50% formamide, 2× SSC (1× SSC = 0.15 mol/L NaCl + 0.015 mol/L sodium citrate), pH 7.0 at 42°C, then three times for 5 minutes in 0.1× SSC at 60°C, followed by a 3-minute wash in 4× SSC, 0.05% Tween 20. The slides were preincubated for 10 minutes with 5% nonfat dry milk in 4× SSC, followed by a 20-minute incubation at room temperature with avidin Texas Red (20 µg/mL [Vector, Burlingame, CA]). The slides were washed twice for 3 minutes in 4× SSC, 0.05% Tween 20, and once in 0.1 mol/L Tris-HCl, 0.15 mol/L NaCl, 0.05% Tween 20 (AB buffer) and incubated for 30 minutes at 37°C with biotinylated goat anti-avidin (Vector) (5 µg/mL) and monoclonal mouse anti-digoxin (Sigma, St Louis, MO) (1:660 dilution) in AB buffer with 1% blocking reagent (Boehringer Mannheim, Germany). After three washes with AB buffer, the slides were incubated for 30 minutes at 37°C with avidin Texas Red (20 µg/mL) and rabbit anti-mouse fluorescein isothiocyanate (FITC) (Sigma) (1:500 dilution) in AB buffer with 1% blocking reagent. After three washes, the final 30-minute incubation at 37°C with goat anti-rabbit FITC (Sigma) (1:2,000 dilution) in AB buffer with 1% blocking reagent was performed. After three 3-minute washes with AB buffer, the slides were mounted in antifade medium, containing 0.3 µg/mL DAPI.

RESULTS

Chromosome 16 cosmids that had been previously isolated were regionally mapped with FISH using metaphase preparations of cell lines from carriers of constitutional rearrangements involving band 16p13, and from eight patients with inv(16) (Fig 1). Among the 99 cosmids tested, four showed a peculiar pattern of hybridization. The cosmids 152, 163, and 175 showed bright signals on 16q22 and two very broad blocks of signals on 16p (Fig 2A). The fourth cosmid, cosmid 177, showed very weak signals on 16q, but very bright blocks on 16p (Fig 2B). In most of the cosmids 177 hybridizations, the signals on 16q could hardly be seen. In addition, all four cosmids also show small signals on the short arm of chromosome 18. Hybridizing and washing under more stringent conditions (60% formamide) produced the same signals, showing close homology between probe and target sequences. Although the cosmids contain inserts of approximately 45 kb (data not shown),
the hybridization signals cover almost half the short arm. In interphase nuclei, the signals of the cosmids 152, 163, and 175 could be seen as two sets of three clusters of distinct dots (Fig 3A). Cosmid 177 showed two sets of two clusters of signals in interphase nuclei (data not shown). No homogeneous painting of certain areas in interphase nuclei can be seen, such as that observed after hybridization with alphoid sequences specific for the centromere region of chromosome 16 (Fig 3B). This leads us to the conclusion that the painted blocks do not consist of contiguous repeated sequences, but are probably clusters of repeats interrupted by other DNA. This is also clearly demonstrated by a double hybridization experiment with cosmid 36, which shows in this metaphase a single signal on 16p in green-yellow, and cosmid 163, which shows large blocks in red, extending beyond cosmid 36 into 16p13 (Fig 4A and B). As cosmid 36 was previously localized proximal to the short arm breakpoints of inv(16) and the t(16;16),5,7 we tested whether the clusters of repeated sequences on 16p spanned these breakpoints. Cosmid 177, which gives very weak signals on 16q, was hybridized to slides containing the inv(16) and t(16;16). In both cases, the distal part of the signal on the short arm was connected to the long arm of chromosome 16, while part of the signal remained on 16p (Fig 2C, D, and E). Illustrations depicting the rearrangements in a schematic way are given in Fig 5. In metaphase spreads of the t(16;16), the transported part of 16p seemed rather small (Fig 2, D and E). However, on these contracted chromosomes, the cosmid 177 consistently showed no signal on the long arm of one chromosome 16, and distinct dots on the other. Therefore, the breakpoints of both inv(16) and t(16;16) must be situated within the distal cluster of repeated sequences. With additional cosmids lacking the repeat sequences and showing single spots in FISH on 16p, the breakpoints of inv(16) and t(16;16) were localized between cosmids C55 and C129 (Fig 1). The differences in size of the hybridization signals on 16q illustrated by the cosmids 152, 163, and 175 and cosmid 177 must be due to a divergence in the repeated sequences present in the different cosmids. Although subcloning of the cosmids and sequencing of the repetitive elements is still in progress, we can assume that at least two repeat units must be involved: one responsible for the bright signals on 16q and one for the combination of broad blocks on 16p with weak signals on 16q and a signal on 18p.

**DISCUSSION**

The serial mapping of cosmids on chromosome 16 showed extensive cross-homology in both the p and q arms of this chromosome. Similar FISH-positive blocks have been observed on chromosome 16 by R.L. Stallings et al (manuscript submitted). While characterizing 3,145 cosmid clones for chromosome 16 by repetitive sequence finger-
printing, 98 cosmids appeared to belong to one very large overlapping set of cosmids (contig). This would seem to suggest the presence of repeated elements in a subset of 4\% to 5\% of the chromosome 16 cosmids, which yields similar restriction fragments in the "fingerprint" analysis. Approximately 1,000 sequence families with a relatively low number of interspersed repeats are believed to be present in the human genome. However, only a handful of the most abundant repeats has been cloned and sequenced. Therefore, the existence of a new class of interspersed repeats clustered on chromosome 16 is not unexpected. We propose that these interspersed repeats facilitate "illegitimate" homologous recombination events leading to the inv(16) and t(16;16). It is clear that repetitive sequences are involved in the origin of some chromosomal rearrangements, such as deletions, translocations, and inversions. In many cases, the recombinations have occurred within, or next to, Alu repeats, while in other cases, local primary or secondary DNA structure facilitates homologous recombination leading to deletions.\textsuperscript{25,26} The origin of an acute nonlymphocytic leukemia with eosinophilic granulatation with inv(16) and t(16;16) is probably similar to myelodysplastic syndrome and t(1;7). In this syndrome, a whole-arm translocation involving the short arm of chromosome 7 and the long arm of chromosome 1 occurs by breakage through the centromeric heterochromatin of both chromosomes.\textsuperscript{31} The resulting monosomy for 7q may contribute to the neoplastic transformation of the cell. For the ANLL M4 eos, characterized by inv(16) or t(16;16), we hypothesize the following sequence of events. Cross-homology between 16p and 16q facilitates rearrangements of the chromosome. If the rearrangement leads to juxtaposition of critical sequences on either arm, a (pre)leukemic cell clone will be formed.

We describe rearrangements involving the distal block of repeats on 16p. It is likely that the homology between 16q and the proximal part of 16p leads to the structural rearrangements too. Presumably, there is no growth advantage provided to a bone marrow cell with a breakpoint at this particular location.

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