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Genetics

Amplified C_λ and c-*abl* genes are on the same marker chromosome in K562 leukemia cells

(chronic myelogenous leukemia/hybridization *in situ*/nonrandom chromosome translocations)

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ABSTRACT **The human leukemia cell line K562, derived from a patient with Philadelphia chromosome-positive chronic myelogenous leukemia, contains amplified c-*abl* oncogenes and unrearranged C_λ genes. Using *in situ* hybridization techniques, we have determined that the amplified c-*abl* and C_λ DNA sequences of K562 cells are both located on the same abnormal acrocentric marker chromosome, which may represent an altered Philadelphia chromosome.**

Since the discovery of the 9;22 translocation in patients with chronic myelogenous leukemia (CML) (1–3), a variety of specific chromosome translocations have been observed in malignancies of the hematopoietic system, including acute promyelocytic leukemia (3), Burkitt lymphoma (4–6), and adult B-cell lymphoma (7). It has been hypothesized that these nonrandom chromosomal aberrations permit the activation of "oncogenes" that initiate neoplastic transformation. For instance, in Burkitt lymphoma patients with the 8;14 translocation, it has been shown that the c-*myc* oncogene translocates to the Ig heavy chain locus situated on chromosome 14 (8–12). Conversely, in Burkitt lymphoma patients with either the variant 8;22 or 2;8 translocations, the Ig light chain genes are brought into juxtaposition with the c-*myc* oncogene located on chromosome 8 (ref. 13 and unpublished data). However, in all of these cases, the c-*myc* oncogene involved in the translocation becomes transcriptionally highly active, whereas the c-*myc* oncogene on the normal chromosome 8 becomes transcriptionally inactive (13–15).

In CML, the human homologue, c-*abl*, of the Abelson murine virus oncogene, v-*abl*, is on the segment of chromosome 9 that translocates to chromosome 22 in the generation of the Philadelphia (Ph) chromosome, and the c-*sis* oncogene, the cellular homologue of the simian sarcoma virus oncogene, is on the segment of chromosome 22 that translocates to chromosome 9 (16–18). At present, it is not clear what role, if any, the c-*abl* and the c-*sis* oncogenes have in the pathogenesis of CML.

A combination of molecular genetic and cytogenetic techniques were used to study the K562 cell line derived from a Ph-positive CML patient for the presence and rearrangements of the C_λ light chain genes that are located on human chromosome 22 (19) and the presence of the c-*abl* oncogene.

MATERIALS AND METHODS

Gel Electrophoresis and Southern Transfer. Agarose gel (0.7% or 1%) electrophoresis was carried out in 40 mM Tris·HCl/5 mM NaOAc/2.0 mM EDTA, pH 8.0. *Hin*dIII-digested phage λ DNA (0.75 μg per lane) (Bethesda Research Laboratories) molecular weight markers were included on every gel. Cellular DNA samples were digested with *Eco*RI and *Hin*dIII restriction endonucleases and then subjected to electrophoresis in a horizontal (Sigma) slab gel (10 μg of DNA per lane). Gels were stained for 10 min with ethidium bromide (1 μg/ml) and photographed under UV light. Transfer of DNA from gel to nitrocellulose sheet (Millipore) was performed essentially as described by Southern (20).

The v-*abl* probe used was the 1.6-kilobase (kb) *Bgl* II–*Bgl* II fragment from 2.5 to 4.1 kilobase pairs on the Abelson oncogene map (21). The C_λ probe was a genomic clone (Chr22λ5) of the C_λ gene in λWes (13). This clone contains an 8.0-kb *Eco*RI fragment that includes Ke-O_z^- and Ke-O_z^+ (13, 22). The DNA probes were labeled with ^{32}P by nick-translation (23) and had specific activities of 0.7–2 × 10^8 cpm/μg of DNA. DNA polymerase I was purchased from Boehringer Mannheim; [α-^{32}P]-NTPs were from Amersham.

Hybridization. DNA on nitrocellulose sheets was hybridized to ^{32}P-labeled probe DNA in a hybridization solution containing 50% (vol/vol) formamide as described (13, 14). After hybridization, the filters were washed, air-dried, and exposed to Kodak XRP5 film for different time periods. Densitometric scanning of bands on x-ray films was carried out by using a Corning 750 scanner.

For blot hybridization, DNA samples obtained from human CML cell lines K562, Bv173 (24), and Nalm1 (25), promyelocytic HL60 cells, human fibroblasts (PAF), and fresh leukocytes of a patient with CML were serially diluted followed by denaturization in 2 M ammonium acetate and 0.8 M NaOH as described (26). The indicated quantities of DNA were then applied to nitrocellulose filters, previously soaked in 3 M sodium chloride/0.3 M sodium citrate, pH 7, and 1 M ammonium acetate by using a "Hybri-Dot" manifold (Bethesda Research Laboratories). The filters were then hybridized to the C_λ probe as described above. After hybridization, the filters were washed twice in 0.5% $NaDodSO_4$/15 mM sodium chloride/1.5 mM sodium citrate, pH 7, at 68°C for 1 hr and then exposed for 3 hr to Kodak XAR5 film at −70°C. The copy numbers of C_λ and c-*abl* genes were determined by densitometric measurements of their respective bands in Southern blots.

Chromosome Analysis and Hybridization *in Situ*. G-banded metaphase chromosomes were produced by the trypsin/Giemsa banding method (27). For *in situ* hybridization studies, air-dried chromosome preparations were initially treated with RNase and then denatured in a 70% formamide (vol/vol)/0.3 M sodium chloride/0.03 M sodium citrate, pH 7, solution at 70°C for 2 min. DNA from C_λ and v-*abl* probes was radioactively labeled with [^3H]dCTP and [^3H]dGTP (New England Nuclear) to a spe-

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Abbreviations: CML, chronic myelogenous leukemia; kb, kilobase(s).

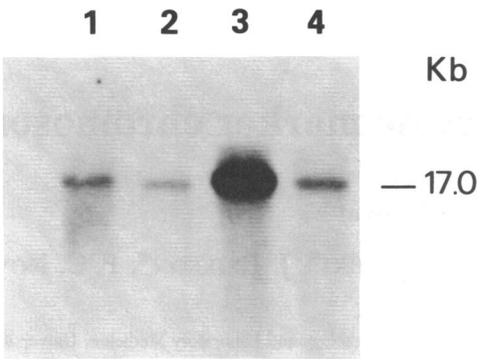

FIG. 1. Southern blotting analysis of *Hin*dIII-digested cellular DNAs by using a v-*abl* DNA probe. Lane 1, Nalm1 DNA; lane 2, P3HRI Burkitt lymphoma DNA; lane 3, K562 DNA; and lane 4, PAF DNA.

cific activity of $2–4 \times 10^7$ cpm/μg of DNA. Radioactively labeled C_λ or v-*abl* probe DNA, diluted to a final concentration of 0.035–0.07 μg/ml, was hybridized to denatured chromosomes for 16 hr at 37°C. After removal of unhybridized DNA by a series of washings, slides were dipped in nuclear track emulsion (Kodak NTB2), stored in lightproof containers at 4°C, and developed 6–10 days later. Chromosomes were G-banded by staining with 0.25% Wright's stain and analyzed microscopically for grain localization by using well-spread metaphases where chromosomal sites with grains were nonoverlapped and identifiable by the presence of G-bands.

RESULTS

c-*abl* and C_λ Genes in K562 Cells. As shown in Fig. 1 (lane 3) and Table 1, K562 cells contain ≈9 copies of the c-*abl* oncogene per haploid genome. No c-*abl* amplification was detected in two other cell lines derived from CML patients in blastic crisis (Fig. 1, lanes 2 and 4, and Table 1). No rearrangements of the c-*abl* gene were detected by *Hin*dIII cleavage of cellular DNA in any of the leukemia cell lines (Fig. 1).

We then examined K562 cells for the presence, rearrangements, and amplification of C_λ genes by Southern blotting analysis to test our hypothesis that the c-*abl* oncogene and the C_λ genes reside in the same amplification unit. This hypothesis was based on the following points: (*i*) the K562 cell line originally contained the Ph chromosome (29); (*ii*) the c-*abl* oncogene, normally located at the distal end of the long arm of chromosome 9 (16), translocates to the deleted chromosome 22 in CML cells; and (*iii*) the C_λ genes remain on the deleted chromosome 22 in CML patients with the 9;22 translocation (unpublished data). As shown in Fig. 2, K562 cells as well as Nalm1 and Bv173 CML cells contain unrearranged C_λ genes that follow the pattern described by Taub *et al.* (28). However, the number of C_λ in K562 cells was ≈9-fold that present in the other two CML cell lines and in human fibroblasts (Fig. 3 and Table 1). Thus, we conclude that the extent of amplification of c-*abl* and of C_λ genes is the same in K562 cells.

Table 1. Amplification of C_λ and c-*abl* genes in K562 cells

Cell line	Copies per haploid genome	
	C_λ*	c-*abl*
PAF	6	1
Nalm1	6	1
Bv173	6	1
K562	52.8	8.7

*Normal human cells contain 6–9 copies of the C_λ genes per haploid genome (22, 28).

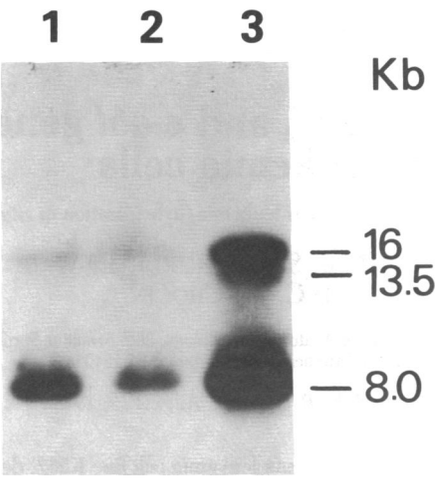

FIG. 2. Southern blotting analysis of *Eco*RI-digested cellular DNAs by using a C_λ probe. Lane 1, Bv173 DNA; lane 2, Nalm1 DNA; and lane 3, K562 DNA.

Chromosomal Localization of the Amplified Sequences. Initial cytogenetic studies revealed that the modal number of chromosomes in the K562 cells maintained in our laboratory is 65. Giemsa-banded karyotyping (Fig. 4) confirmed that these cells contain some of the marker chromosomes previously reported (29) but lack both the Ph chromosome and the chromosome 9 resulting from the t(9;22) described initially (29).

In situ hybridization was performed on metaphase chromosome preparations of K562 cells to determine the chromosomal location of the amplified C_λ and v-*abl* sequences. Fig. 5 illustrates representative autoradiographs of portions of metaphase spreads from K562 cells hybridized with either C_λ or v-*abl* DNA probes. As indicated, heavy labeling with each probe was consistently limited to a morphologically distinctive acrocentric marker chromosome, comparable in size to E or F group chromosomes. We examined 50 metaphases with chromosomally localized grains (13/50 hybridized with C_λ and 37/50 hybridized with v-*abl*). In these metaphases, the majority

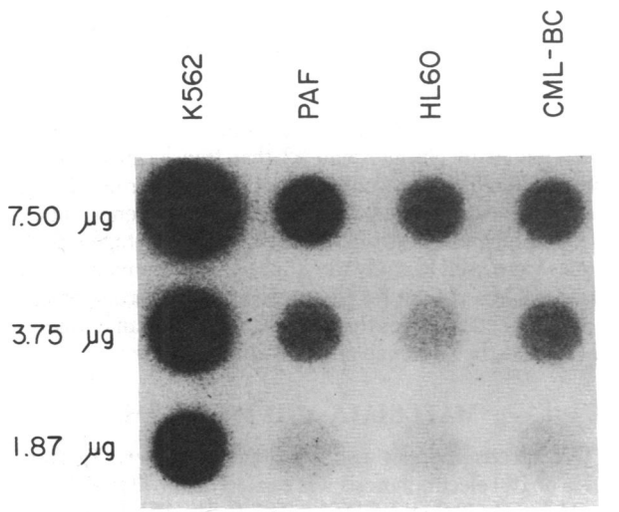

FIG. 3. Estimation of c-*abl* gene amplification in K562 human leukemia cells. The indicated amounts of DNA derived from K562 cells, PAF human fibroblasts, HL60 human promyelocytic cells, and a fresh human chronic myelogenous leukemia (CML-BC) were dot-blotted to a nitrocellulose filter that was hybridized to the v-*abl* probe.

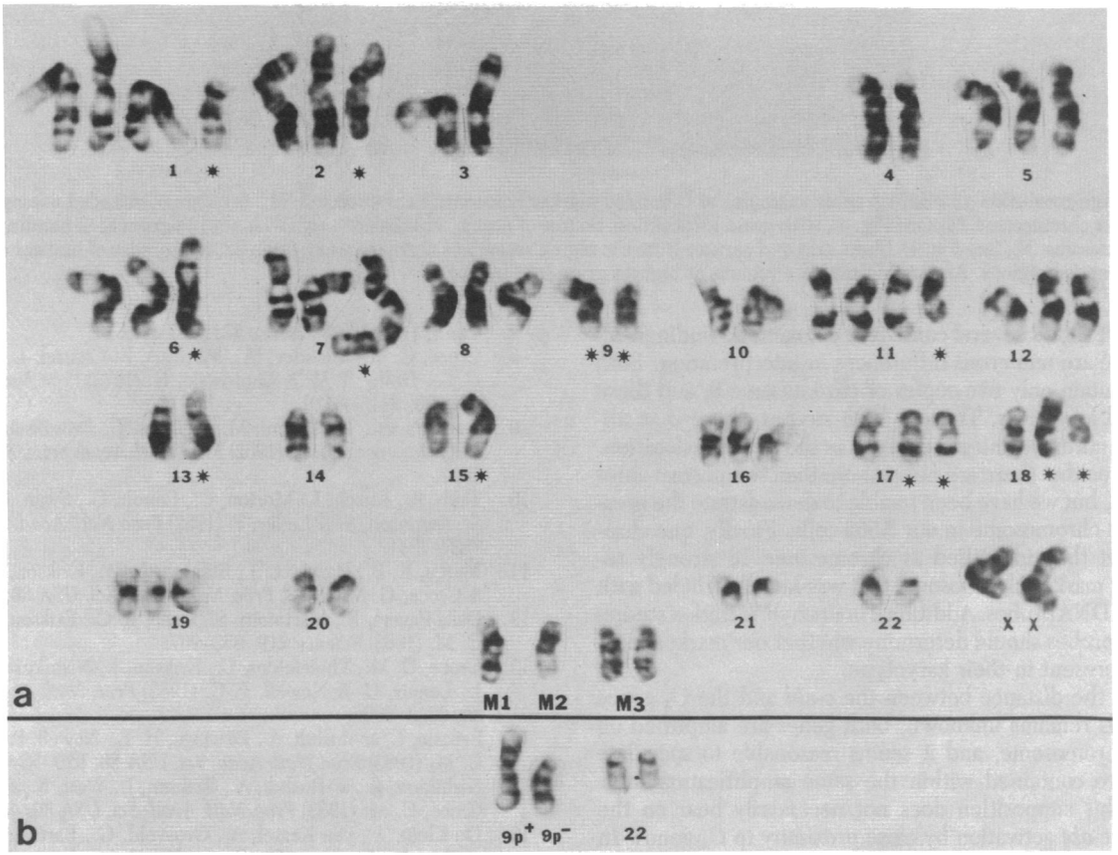

FIG. 4. Giemsa banded chromosomes of K562 cell line. (*a*) Representative karyotype, including 12 abnormal chromosomes indicated by * and three markers, M_1, M_2, and M_3. Two copies each of M_1 and M_3 were present in over 50% of the cells studied. (*b*) Pairs of Giemsa banded chromosomes 9 and 22 from another K562 metaphase. Our cell line does not now contain a typical Ph chromosome or $9q^+$.

of all grains was concentrated on the marker chromosome. However, precise quantitation was not possible due to the heavy accumulation of grains (see Fig. 5). Fig. 6 presents three examples of the labeled marker chromosome selected for visibility of its G-banding pattern. A close comparison of these chromosomes with the G-band idiogram of the M_3 marker (see Fig. 6) indicates that they are one and the same. Although the short arm and paracentromeric regions of this marker chromosome have a G-banding pattern consistent with that observed for chromosome 22, the remainder of the long arm cannot be identified as being from any normal chromosome. Such abnormally banded regions have been reported to be sites of gene amplification (31), as have the more commonly observed homogeneously staining regions and double minute chromosomes (30, 31).

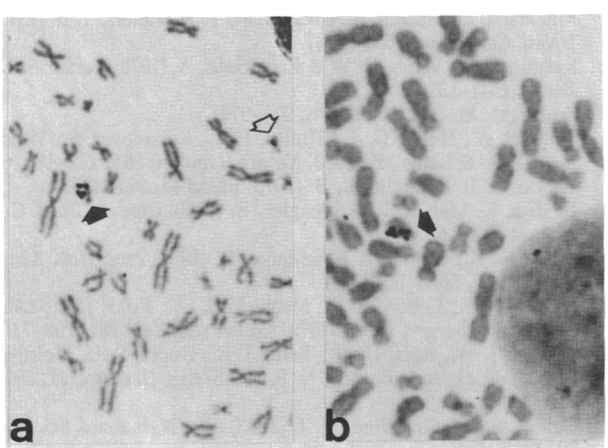

FIG. 5. Partial K562 metaphases demonstrating hybridization with ^{3}H-labeled C_λ (*a*) and v-*abl* DNA (*b*) probes. In both instances grains are clustered over the acrocentric marker, M_3 (black arrowheads). Note grain indicating C_λ probe hybridized to normal 22 (clear arrowhead).

Grains from the C_λ and v-*abl* DNA probes generally were localized over two segments of the abnormally banded regions, one closer to the centromere and the other closer to the telomere (Figs. 4 and 5), suggesting the presence of two duplicated areas of gene amplification on this marker chromosome. Fig. 6 shows an idiogram of the banding pattern of the marker chromosome and delineates the two regions of highest autoradiographic signal.

DISCUSSION

The karyotype of the K562 cell line we examined closely resembles that recently reported by several laboratories detailing the presence of multiple marker chromosomes with or without the continued presence of the Ph chromosome (22, 32, 33). Thus, it is tempting to conclude that the Ph chromosome persists in K562 cells within the marker chromosome that was heavily labeled by the C_λ and v-*abl* DNA probes. Additional insight into this marker chromosome that has arisen *in vitro* could be accomplished by employing DNA probes specific for other regions of chromosome 22 because they would delineate the extent to which this marker is derived from chromosome 22.

A recent report described the amplification of c-*abl* and C_λ sequences in a K562 cell line (34). Comparison of the karyotype of the K562 cell line used in that study with the one described

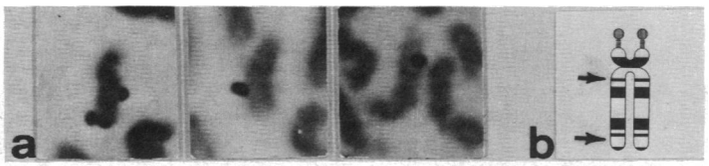

FIG. 6. High-resolution autoradiographic examples of G-banded marker acrocentric chromosome, M_3. (*a*) Three G-banded examples of acrocentric marker chromosome, M_3 (see Fig. 4), with grain localization. Source of probe, ^{3}H-labeled v-*abl* DNA. (*b*) Idiogram of G-banding pattern of marker chromosome, M_3 (see Fig. 4). Short arm and paracentromeric region resembles normal chromosome 22. Remainder of marker composed of abnormally banded regions. Arrows indicate two regions of highest grain localization.

in this report shows several consistent cytogenetic findings, although there are numerous differences in interpretation. Both cell lines contain only two copies of chromosome 9, and these have normal long arms. Thus, in both studies, there is an absence of the chromosome usually seen in the 9;22 translocation. Also, we agree that there are never more than two normal chromosomes 22, but we have been unable to demonstrate the presence of a Ph chromosome in our K562 cells. Finally, one chromosome that they identified as chromosome 13 strongly resembles our marker chromosome that was heavily labeled with v-*abl* and C_λ DNA probes. Additional *in situ* hybridization studies using these probes should determine whether our marker chromosome is present in their karyotype.

Although the distance between the c-*abl* and the C_λ genes in K562 cells remains unknown, both genes are amplified on the same chromosome, and it seems reasonable to speculate that they are contained within the same amplification unit. However, this supposition does not necessarily bear on the question of c-*abl* activation by close proximity to C_λ genes. In fact, several notable differences exist between the present findings and those obtained in analysis of Burkitt lymphoma cells with the 8;22 translocation (13). First, Burkitt lymphoma cells belong to the B-cell lineage and are capable of expressing Ig genes, whereas CML cells are typically differentiated along the myeloid cell lineage and do not normally express Ig genes. Second, in Burkitt lymphoma cells, the C_λ genes translocate from chromosome 22 to chromosome 8 (13, 28), whereas in CML cells, the C_λ genes remain on chromosome 22 (28). Lastly, in Burkitt lymphoma cells, one of the translocated C_λ genes is rearranged (13), whereas we were unable to detect rearrangements of the C_λ genes in CML cells.

Nevertheless, because the C_λ and the c-*abl* genes are likely to be in the same amplification unit in K562 cells, it should be possible to exploit C_λ and c-*abl* DNA probes to isolate and characterize the DNA sequences adjacent to the breakpoint. This information might, in turn, prove useful in exploring the molecular mechanisms resulting from the 9;22 reciprocal translocations that are involved in the pathogenesis of CML.

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