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DNA ANALYSIS USING LONG-TERM PRESERVED FIXED CYTOGENETIC PREPARATIONS

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Summary DNA was isolated from cell pellets that had been stored in methanol–acetic acid (3 : 1) at −20°C up to 6 years. These cell pellets contained high molecular weight DNA sufficient for Southern blot hybridization. The method enables us to study the DNA of a patient after completing chromosome analysis or in cases where DNA samples are otherwise not available.

Key Words high molecular weight DNA, DNA extraction, chromosome preparation, Southern blotting, breakpoint cluster region

INTRODUCTION

DNA analysis is now an important tool to study hematologic diseases (Arnold *et al.*, 1983; Groffen *et al.*, 1984; Kurzrock *et al.*, 1986). However, DNA extraction from fresh blood cells or tissue samples was not performed routinely until recently. Barker *et al.* (1986) first described a method for extracting DNA from fixed cytogenetic cell pellets stored up to 1.5 years. We extracted DNA from long-preserved fixed cytogenetic material, on which chromosome analysis had been carried out previously; the remainder of the cell pellets had been kept in the methanol acetic acid mixture (3 : 1) at −20°C up to 6 years. The purity and the degree of degradation of the DNA were examined. DNA obtained from patients with chronic myelogenous leukemia (CML) was digested, electrophoresed, and hybridized to detect rearrangement within the breakpoint cluster region (bcr).

MATERIALS AND METHODS

Patients, culture method, procedure for chromosome preparation, and storage of

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cell pellet. The material consisted of blood or bone marrow cells obtained from 19 patients with hematologic malignancies karyotyped in our laboratory; 14 patients with Ph^1-positive CML, four with Ph^1-negative CML, and one with Ph^1-positive acute leukemia. The cells were cultured at 37°C in RPMI 1640 medium (GIBCO) supplemented with 15% fetal bovine serum for 24 hr without mitogen and colcemid was added 1 hr before harvesting. After hypotonic treatment (0.075 M KCl for 20 min at 37°C) the cells were fixed with a 3 : 1 (v/v) mixture of methanol and glacial acetic acid. After the chromosome preparations were made, the rest of the cells were stored at −20°C in the same fixative. The storage term was 70 months for one sample, 60 months for two, 40 to 59 months for four, 30 to 39 months for two, 20 to 29 months for two, 10 to 19 months for four, and less than 10 months for four.

DNA extraction. After the fixative was removed, each cell pellet was washed several times with absolute ethanol and resuspended in TNE (20 mM Tris-HCl at pH 7.4, 10 mM NaCl, 0.1 mM EDTA). DNA was isolated with proteinase K and phenol/chloroform treatment by the procedure described by Maniatis *et al.* (1982) and Barker *et al.* (1986). The absorbance ratio was estimated for the purity of the DNA samples. DNA extracted from 20 fresh blood samples was used as a control.

Southern blotting. Genomic DNA was digested with restriction enzymes (*Bgl*II, *Bam*HI, *Hin*dIII, or *Eco*RI), electrophoresed in 0.8% agarose gels, and transferred to nylon membranes (Biodyne, Pall, Glen Cove, N.Y.) by the method of Southern (1975). A 3′-*bcr* genomic probe (Pr-1, Oncogene Science, Inc., Mineola, N.Y.) and a 5′-*bcr* cDNA probe (Pr-2, Oncogene Science) were used. The probes were labeled by the multiprime labeling method (Amersham RPN1601) to a specific activity of approximately 1×10^9 cpm/μg and hybridized. The filters were autoradiographed on Kodak XK-1 films with intensifying screens at −80°C for 16–72 hr.

RESULTS

The DNA was successfully isolated from all the fixed cell pellets. The average yield of DNA extracted was 90.7 μg per 0.1 ml of pellet volume. The mean absorbance ratios at 260/280 nm and 260/230 nm were 1.85 and 2.36, respectively, which did not differ from those of DNA obtained from fresh blood samples (Table 1). Moreover, the ratios for the DNA extracted from material that had been kept more than 3 years did not differ from those from material stored for less than 3 years.

The undigested DNA samples were electrophoresed in 0.7% agarose gel and stained with ethidium bromide. The DNA extracted from the fixed pellets was partially degraded to various degrees but contained high molecular weight DNA (larger than 23 kb); the degree of degradation appeared to be correlated with the term of storage (Fig. 1). Figure 2 shows the results of Southern blotting. All the DNA samples could be digested with restriction enzymes, successfully hybridized to the *bcr* probes, and revealed clear autoradiographic bands similar to control DNA. However, when compared to the control DNA, it appeared that the DNA

Table 1. Absorbance ratio and yield of DNA isolated from stored cell pellets.

Specimen	260/280 nm (mean ± SD)	260/230 nm (mean ± SD)	μgDNA/0.1 ml cell pellet	Number of samples
Fixed pellets [a]				
≧3 yrs	1.81±0.09	2.32±0.65	107.4±55.6	8
<3 yrs	1.89±0.22	2.39±0.63	78.5±44.2	11
Cells, fresh	1.80±0.08	2.31±0.18	—	20

[a] Cells stored more than 3 years, or less than 3 years.

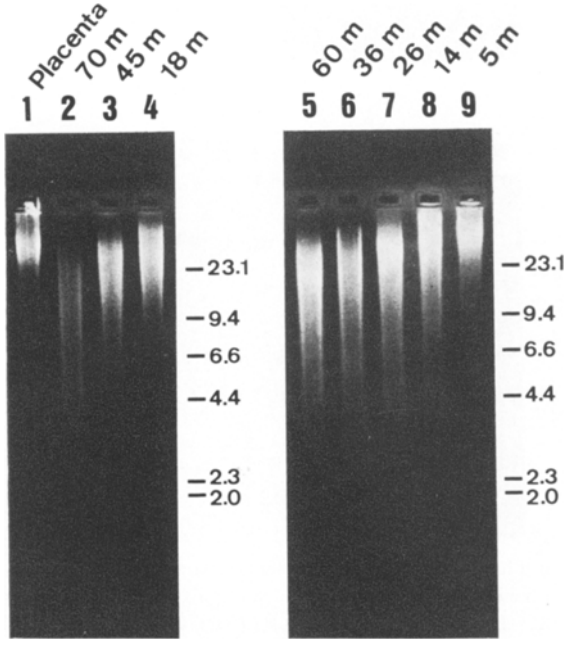

Fig. 1. Agarose gel (0.7%) electrophoresis of undigested DNA isolated from stored cell pellets. Lane 1, placental DNA (control); lanes 2–9, DNA extracted from stored cell pellets. Length of storage is indicated in months at the top. Molecular weight markers, given in kilobase pairs, were lambda phage DNA digested with *Hin*dIII.

samples from the fixed cell pellets were somewhat difficult to digest completely with restriction enzymes. We examined a *bcr* rearrangement in DNA samples extracted from fixed cell pellets in three CML patients with variant Ph^1, one CML with two Ph^1 chromosomes, and four Ph^1-negative CML. As is shown in Fig. 2 (A, lane 2), the DNA stored for 60 months has a rearranged *bcr* gene. Two cases of variant Ph^1 (stored for 70 months and 50 months, respectively) reveal no rearrangement when digested with *Eco*RI (Fig. 2B, lanes 2, 3). In a patient with CML, we examined

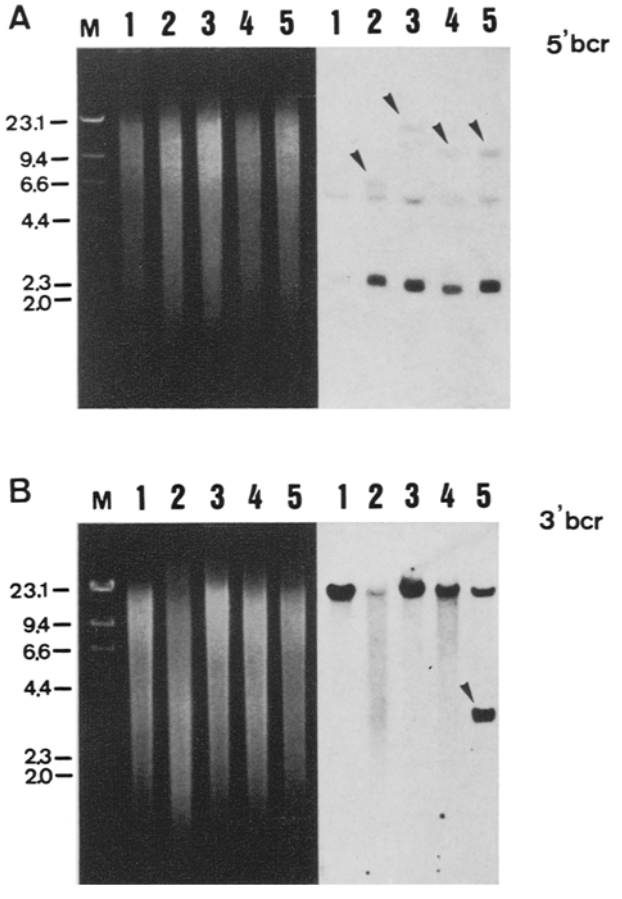

Fig. 2. A: Electrophoresis of DNA samples digested with *Bgl*II in 0.8% agarose gel and stained with ethidium bromide (left). Southern transfer, hybridized to 5′*bcr* probe (right). B: DNA digested with *Eco*RI (left), and hybridized to 3′*bcr* (right).

A: Lane 1, placental DNA (control); lane 2, CML with t(9;22;12) (60-month-old pellet); lane 3, CML with standard Ph^1 (9-month-old); lane 4, chronic phase CML with standard Ph^1 (fresh marrow); lane 5, accelerated phase of the same patient as lane 4 showing double Ph^1 (10-month-old).

B: Lane 1, placental DNA; lane 2, CML with t(4;18;13;9;22) (70-month-old pellet); lane 3, CML with t(9;22;15) (50-month-old); lane 4, the same case as A-2 (60-month-old); lane 5, CML with a complex Ph^1 involving #9, #22, and #10 (fresh marrow). M, lambda phage DNA digested with *Hin*dIII. Dashes indicate germ-line bands and arrows indicate rearranged bands.

the *bcr* rearrangement using the 5′-*bcr* probe (Pr-2) in two DNA samples, one obtained in the chronic phase from fresh blood cells having a standard Ph^1 and the other in the accelerated phase from 10-month-old fixed cell pellet having double

Ph^1 in all the metaphases examined. As is shown in Fig. 2 (A, lanes 4, 5), the same *bcr* rearrangement is detected.

DISCUSSION

Our results demonstrate that the DNA extracted from a cell pellet kept for up to 6 years at −20°C in a methanol acetate mixture preserves high molecular weight DNA although the chromosome preparation procedure was done at room temperature. The method for extracting DNA from a cell pellet is essentially the same as that used for fresh cells or tissues. Although the isolated DNA contains partially degraded DNA, a sufficient amount of high molecular weight DNA is still contained for Southern blot analysis. The extent of degradation appears to be correlated with the length of storage.

A clonal rearrangement of the *bcr* gene can be detected using the DNA isolated from mitotic cell pellets of patients with Ph^1-positive CML. Thus, the detection of gene rearrangement is possible for specific samples after completing chromosome analysis or *in situ* hybridization studies as occasion demands.

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