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CASE REPORT

AMPLIFICATION OF c-*myc* AND pvt-1 HOMOLOGOUS SEQUENCES IN ACUTE NONLYMPHATIC LEUKEMIA

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Abstract—Leukemic cells with double minute (DM) chromosomes from an ANLL(M1) patient were found to carry 10–15 fold amplified c-*myc* sequences. The linked pvt-1-like locus was amplified at the same level, suggesting that the c-*myc* amplicon is at least 300 kb in size.

Key words: c-*myc*, amplification, leukemia.

INTRODUCTION

ONCOGENE amplification is frequently found in certain tumor cells or in tumor-derived cell lines [1]. Commonly, the amplification of oncogenes does not affect the majority of tumors of any given histological type. When it does, however, it occurs as a variable and often late progression associated event [1]. In general, amplification of genes is maintained in tumors and derived cell lines only in cases where it provides the cell with a selective growth advantage [1]. It may act by decreasing the dependence of the cell on limiting growth factors or by increasing its sensitivity to them. The majority of the documented cases of oncogene amplification in human tumors concerns the members of the *myc* family (see [1]). Progression related amplification of one or several alternative *myc* proto-oncogenes was found in plasma cell leukemia, small cell lung carcinoma, neuroblastoma and plasma cell leukemia (see [1, 2]). We here report the appearance of double minute chromosomes (DMs) and the correlated amplification of c-*myc* and pvt-1 in tumor cells from a patient with Acute Non-Lymphatic Leukemia (ANLL) of FAB class M1. The coamplification of the c-*myc* linked pvt-1 locus defines the minimum size of the amplicon to be 300 kb.

Abbreviations: *ANLL*, acute non-lymphatic leukemia; *DM*, double minute chromosome; *WBC*, white blood cell.

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CASE REPORT

A 58-year old man was admitted with anemia, thrombocytopenia and severe bleeding from the upper gastrointestinal tract. Physical examination revealed diffuse purpuric effusions and sternal tenderness. The blood count showed Hgb 6.5 g/dl, platelets 18×10^9/l, WBC 140×10^9/l with more than 95% blast cells. Bone marrow aspirate showed 100% blasts. The diagnosis was according to the FAB-classification ANLL, subtype M1. The patient died a few hours after admission.

MATERIALS AND METHODS

Cytogenetics

Chromosome analysis was performed on bone marrow cells immediately after collection.

Isolation of DNA and Southern hybridization

High-molecular DNA was isolated and cleaved with restriction enzymes as described previously [2]. Agarose gel electrophoresis was carried out in 89 mM Tris-borate pH 8.2 and 2 mM EDTA in a horizontal 0.8% agarose gel. Gels were stained and photographed, and the DNA fragments were transferred to nitrocellulose membranes. Human c-*myc* DNA (pMC41-RC) [3], c-*mos* genomic clone [4] and rat mis-1 DNA [5] were labelled with P-dCTP by nick translation to a specific activity of 5×10^8–10^9 counts/min/μg and hydridized to EcoRI cleaved DNA blotted to nitrocellulose filters as described previously [2]. Intensities were quantitated with an LKB laser densitometer. The amplification was computed by dividing the signal intensity from ANLL DNA by the signal intensity obtained with the same probe in the same hybridization assay on human placenta DNA.

RESULTS AND DISCUSSION

Cytogenetic analysis revealed a large number of DMs (from 12 to 28 per cell) in 80% of the cells. The karyotype was hypodiploid, with a number of abnormalities: 45, X,−Y,+5,−8, del(9) (q13q32), −10, −17, +m1, +m2, +DMs (Fig. 1). DNA was isolated from sternal bone marrow, and 5 μg was cleaved with EcoRI, electrophoresed and tested for the amplification of the proto-oncogenes *mos*, *myb*, N-*myc*, *abl*, *sis* and c-*myc* by Southern hybridization (data not shown). Only c-*myc* was amplified. To define the level of amplification, 5, 2.5 and 1.25 μg of ANLL and human placenta DNA was cleaved with EcoRI and analysed by Southern hydridization. The densitometric analysis of the autoradiograms indicated a 10–15 fold amplification of the c-*myc* gene (Fig. 2A). Another chr.8 specific DNA probe, c-*mos*, showed uniform labelling of a 9.6-kb long fragment, both with ANLL and human placenta DNA (Fig. 2B). The amplification was thus specific for the c-*myc* gene. In order to gain some information about the size of the c-*myc* amplicon, ANLL DNA was hybridized to the rat mis-1 DNA probe [5] known to detect the human pvt-1 locus [6]. Hybridization of the mis-1 DNA probe with ANLL DNA showed an approximate 10–15 fold amplification of the normal 12.5 kb EcoRI fragment carrying the pvt-1 locus (Fig. 2C).

The distance between the human c-*myc* and the pvt-1 region has been estimated to be at least 300 kb [6]. The coamplification of c-*myc* and pvt-1 suggests that the size of the amplicon must be longer than 300 kb. Why would c-*myc* and pvt-1 amplification confer a selective advantage of an ANLL clone? The c-*myc* gene encodes a nuclear protein that appears to be involved in DNA synthesis [7]. In resting lymphocytes and fibroblasts c-*myc* is not expressed, but can be readily induced by mitogens. Transfection of inducible cells with *myc* expressing constructs may prevent their entry into a terminal differentiation pathway (see [8]). This suggests that interference with the normal level of c-*myc* expression can block the programmed transition of the cell to the resting state and may thereby contribute to tumor development. It is noteworthy that four of the six c-*myc* amplicons so far studied have involved the pvt-1 region. This region is frequently rearranged by retroviral insertion in murine T-cell lymphomas, by chromosomal translocation in murine variant plasmacytomas as well as in the variant translocation carrying Burkitt lymphomas [5, 6]. This suggests that the rearrangement/amplification of the pvt-like region may contribute to the process of tumorigenesis in certain cell types. The effect of the pvt-1 rearrangements on the c-*myc* expression has not yet been clarified. The pvt-1 region might bear a gene that can regulate myc expression in *trans*. Transcripts from pvt-1, however, have not yet been identified. Alternatively, pvt-1 alterations may act in *cis* via perturbations of long-range chromatin folding, since the c-*myc* and pvt regions belong to the same functional unit of chromatin architecture. Disturbance of this functional unit may affect the expression of the c-*myc* gene.

In Small Cell Lung Carcinoma (SCLC) the amplification of c-*myc* is correlated with the appearance of a variant phenotype with a faster growing tumor clone, increased cloning efficiency and more malignant features *in vivo* than the original prototype [1]. The c-*myc* gene is also amplified in some plasma cell leukemias, but not in multiple myeloma [2]. Although sporadic, c-*myc* amplification in the plasma cell leukemias [2] and the ANLL case reported here may promote the clonal evolution of a more highly malignant cell variant.

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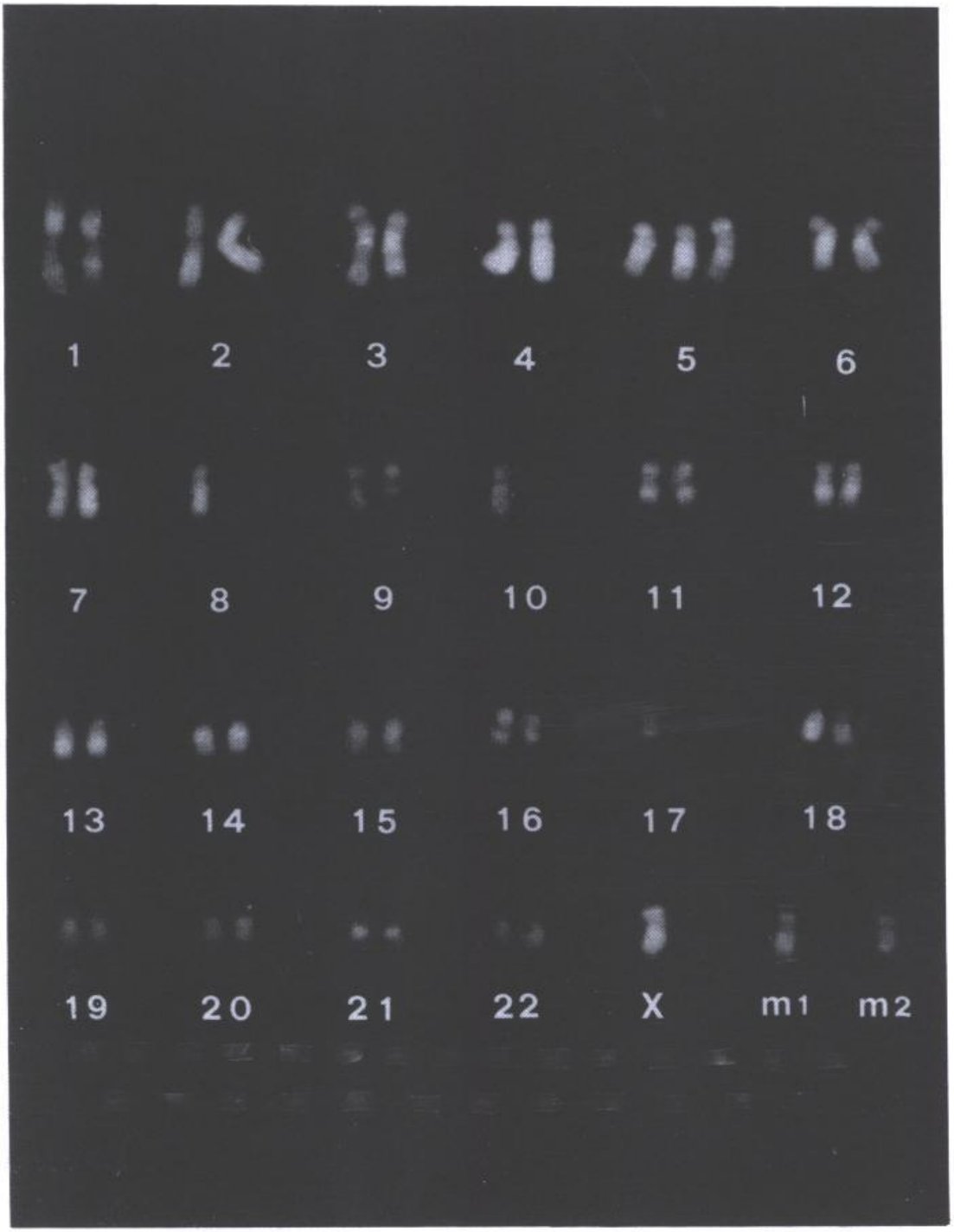

FIG. 1. Representative karyotype of bone marrow cell from ANLL. Twenty cells were karyotyped and Q banded according to standard techniques. This cell contains 27 copies of double minutes. The presence of double minutes in more than 20 copies was evident in all twenty cells karyotyped.

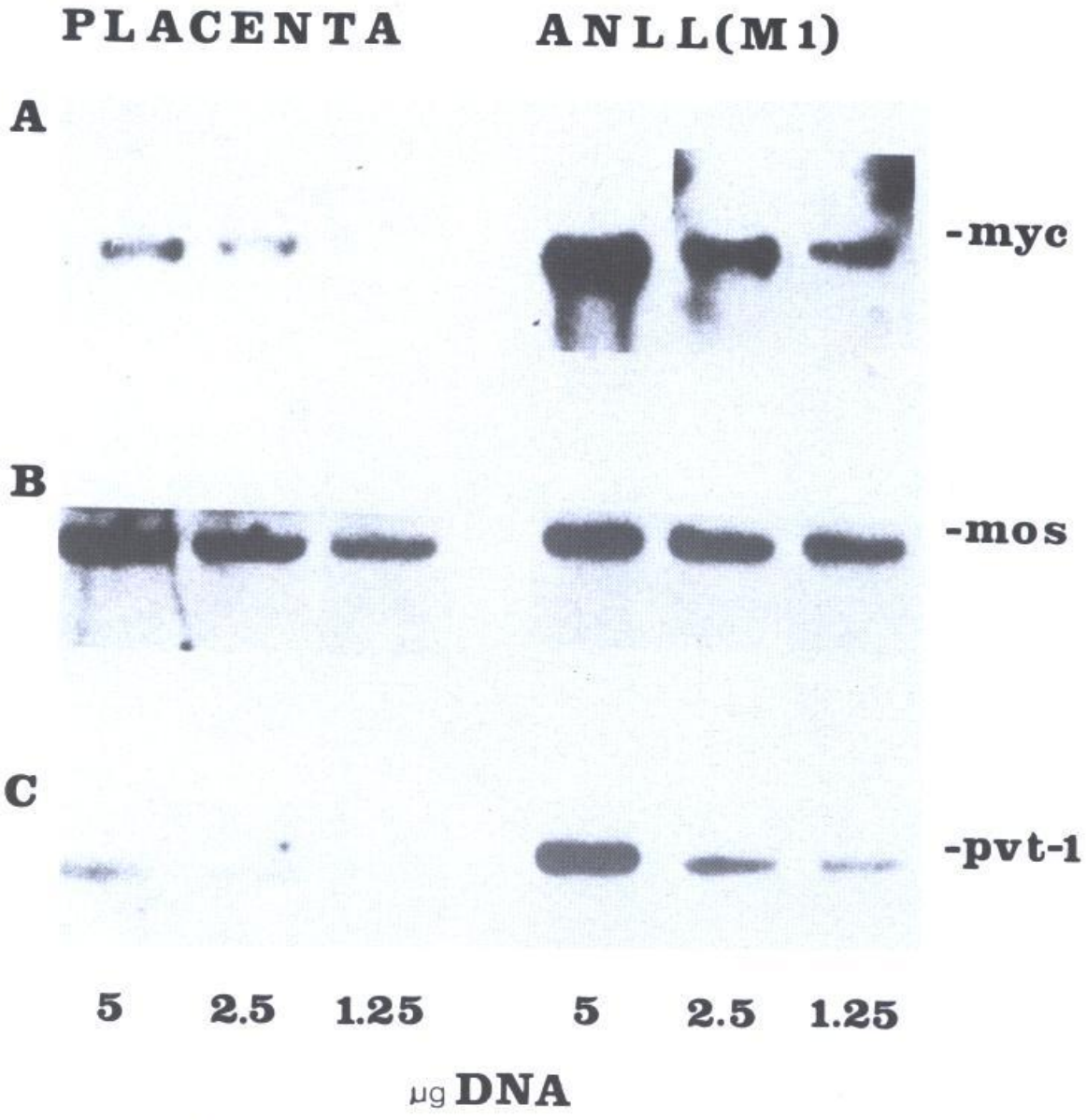

FIG. 2. Southern analysis of DNA from ANLL and human placenta cells. 5, 2.5 and 1.25 μg of DNA of ANLL cells and human placenta was cleaved with EcoRI (A, C) and BamHI (B) enzymes and electrophoresed and blotted to nitrocellulose filters as described in Materials and Methods. The blots were hybridized to ^{32}P-labeled human c-*myc* (pMC41-RC) (Fig. 2A), c-*mos* (p-HA2) (Fig. 2B) and rat mis-1 (Fig. 2C) DNA probes in a condition as described previously [3].

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