Establishment of a Hybrid Cell System Between Malignant Burkitt's Lymphoma Cells and Nonmalignant Lymphoblastoid Cells

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A. Introduction

Burkitt's lymphoma (BL) is a high-grade malignant B-cell lymphoma found in a high-incidence endemic form in equatorial Africa and a rare sporadic form [1]. This tumor is strongly associated with a viral infection and specific chromosomal translocations. In the vast majority of endemic BL, Epstein-Barr virus (EBV) DNA has been demonstrated [2]. In all BLs one of three specific translocations is present, involving the cellular oncogene c-*myc* on chromosome 8 and loci for immunoglobulin genes on chromosomes 14, 22, and 2 [3]. The deregulation of c-*myc* caused by these translocations and the EBV infection are thought to be the critical steps in the development of EBV-positive BL, although the precise mechanisms are still unclear.

To investigate whether defects in cellular control genes may also play a causal role in BL pathogenesis, we established a fusion cell system between malignant BL cells and nonmalignant EBV-immortalized lymphocytes. The fusion of malignant and nonmalignant cells and the suppression of the tumorigenic phenotype in the resulting somatic cell hybrids, demonstrated for many tumors, but not yet for lymphoma-lymphocyte hybrids, has led to the concept of tumor suppressor genes [4, 5]. The activity of these genes is thought to prevent the malignant transformation of a normal cell and to suppress the malignant phenotype in somatic cell hybrids.

B. Results

The following cell lines (established and kindly provided by G. Lenoir, IARC, Lyon) were used: BL60, an EBV-positive BL cell line with a (8,22) translocation and highly tumorigenic in nude mice; and IARC 277, an EBV-immortalized nontumorigenic lymphoblastoid cell line originating from the same patient.

The neomycin resistance plasmid pSV2neo was introduced into BL60 cells by electroporation [6]. Several sublines resistant to 1200 μg/ml of the neomycin analogue G418 were obtained, which showed genomic integration of pSV2neo in Southern blot analysis. One of these sublines was cultured in medium containing 10^{-5} *M* 6-thioguanine (6TG) to select for spontaneous hypoxanthine-guanine-phosphoribosyltransferase (HGPRT)-negative mutants. A 6TG-resistant subline, named P7, that was stably HGPRT-negative (demonstrated by the consistent failure of growing in hypoxanthine, aminopterine, thymidine (HAT) medium) and in addition G418 resistant, was obtained. After ensuring that the high tumorigenicity of BL60 in nude mice was maintained in P7, this subline was used in fusion experiments with IARC 277.

The cells were attached to the bottom of plastic petri dishes by concanavalin A [7] and subsequently fused by PEG 1500. Thirteen hybrid clones growing in HAT medium containing G418 were obtained

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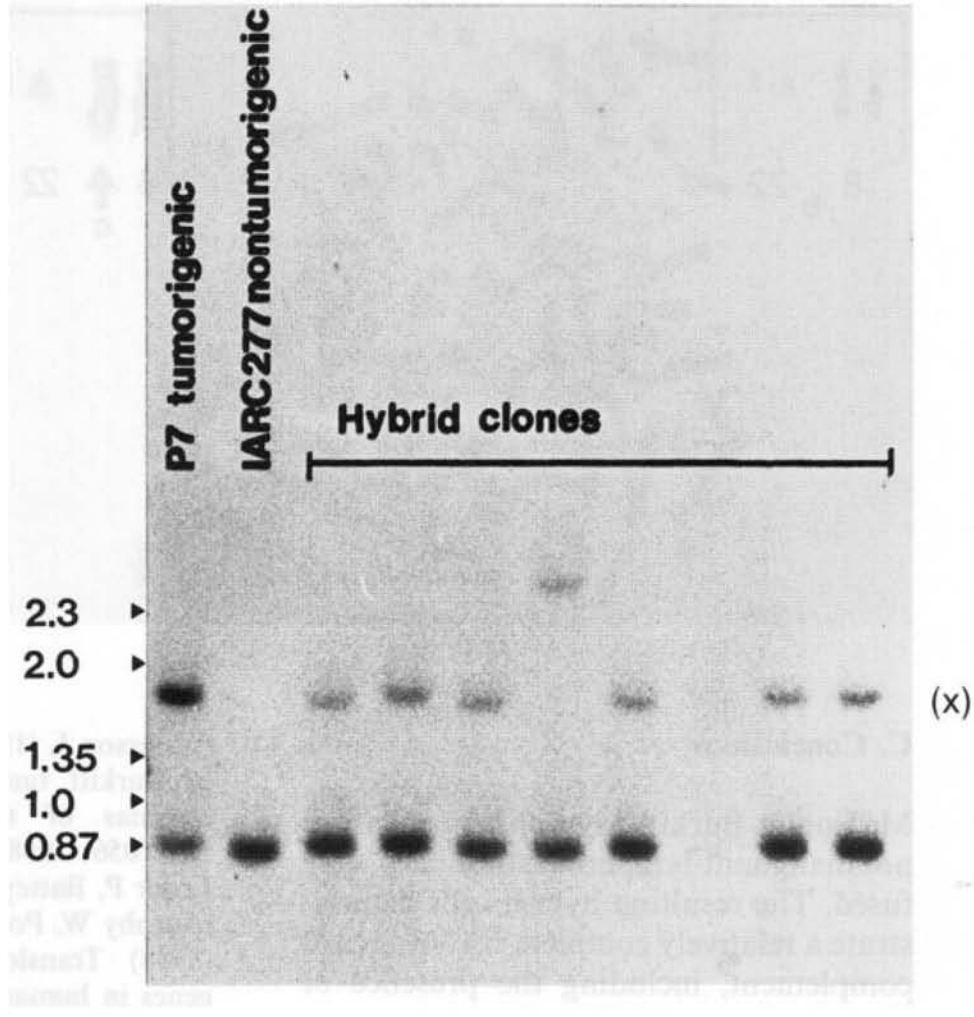

Fig. 1. Southern blot analysis for demonstration of the c-*myc* *Pvu*II-RFLP: presence of an additional DNA fragment (*X*) in the malignant BL60 subline P7 and in the hybrid clones in contrast to the nonmalignant cell line IARC 277, indicating the presence of the t(8;22) translocation. *Pvu*II-digested DNA was hybridized with c-*myc* exon 1 probe

in two independent experiments. The hybrid nature of these cells was verified by flow cytometry, restriction fragment length polymorphism (RFLP) analysis, and cytogenetics. Flow cytometric analysis of the DNA content revealed that the hybrid clones were near tetraploid by comparison with the diploid nontumorigenic and the near diploid tumorigenic parental line. For RFLP analysis cellular DNA was digested with the restriction enzyme *Pvu*II and probed in Southern blot analysis with radioactive-labeled c-*myc* exon 1. Because on chromosome $8q^+$, involved in the (8/22) translocation of BL60, one *Pvu*II site is abolished in c-*myc* exon 1, a new DNA fragment is created additional to the germline fragment [8]. This RFLP was used to demonstrate the presence of one chromosome $8q^+$ versus three normal chromosomes 8 in the hybrid cells (see Fig. 1).

These results were confirmed by cytogenetic analysis. Up to the present time seven hybrid clones have been analyzed. All show a near tetraploid karyotype with a modal range of 82–93 (peak 87) without any significant differences among each other (5 months after fusion). For all clones the presence of one copy of chromosome $8q^+$ could also be demonstrated in agreement with RFLP analysis (see Fig. 2).

We are currently investigating these hybrid clones with regard to the parameters of malignancy in vitro and in vivo. Preliminary results indicate a suppression of the BL phenotype in the hybrid cells demonstrating the same growth pattern as the nonmalignant IARC 277 cells, e.g., clumping in suspension culture, similar growth rate and maximal cell density. While the BL cells from large, progressively growing tumors without any sign of regression in nude mice, the grafts of the hybrid clones tested so far, as well as of the nonmalignant IARC 277 cells, stop growing after reaching a maximal size of 1 cm diameter, and then undergo complete regression.

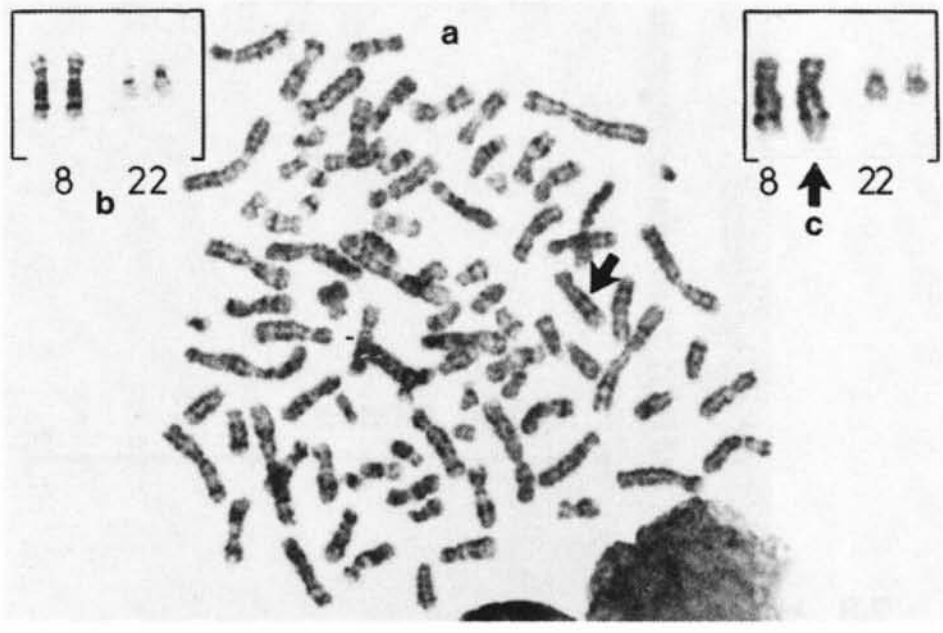

Fig. 2. **a** Cytogenetic demonstration of the t(8;22) translocation in the metaphase of one hybrid clone (*arrow*). **b** Normal chromosomes 8 and 22 of the nontumorigenic cell line IARC 277. **c** t(8;22) translocation of Burkitt's lymphoma subline P7 (*arrow*)

C. Conclusions

Malignant Burkitt's lymphoma cells and nonmalignant lymphoblastoid cells were fused. The resulting hybrid cells demonstrate a relatively complete chromosomal complement, including the presence of the (8,22) translocation, which is thought to play a causal role in the process of malignant transformation. First experiments indicate the suppression of the BL phenotype in the fusion cells. We hope that these hybrid cell system will be useful for further characterization of the mechanisms leading to the development of BL especially with regard to a possible causal role of defects in cellular suppressor genes.

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