Translocation (8;21) in Acute Nonlymphocytic Leukemia Delineated by Chromosomal In Situ Suppression Hybridization

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ABSTRACT: *In situ suppression hybridization with recombinant bacteriophage DNA libraries for chromosomes 8 and 21 was performed in two cases of acute nonlymphocytic leukemia, type FAB M2. In both cases, cytogenetic analysis by conventional G-banding revealed t(8;21)(q22;q22). In situ suppression hybridization was able to prove the reciprocal nature of the translocation in both cases by identifying the terminal end of chromosome 21 translocated to the derivative chromosome 8q−.*

INTRODUCTION

Nonrandom chromosomal anomalies, in addition to phenotyping, have been well accepted as an important feature in the subclassification of acute nonlymphocytic leukemias (ANLL). Therefore, it was proposed to replace the conventional FAB (French, American, British) nomenclature [1] with a new convention, designated as the MIC classification, which would include morphology, immunophenotyping, and chromosomal anomalies [2]. Even though the significant impact of chromosomal findings for prognosis has been accepted, their broader application in clinical practice has been hampered by the high expenditure necessary for detailed banding studies, which are time consuming, need skilled personnel, and, so far, cannot be automated. Therefore, there remains a strong demand for new techniques to simplify, accelerate, and improve the detection of specific chromosomal anomalies in malignancies.

A recent modification of non-radioactive in situ hybridization (for review, see [3]), called chromosome painting [4] or chromosomal in situ suppression (CISS) hybridization [5], has provided a new tool with which to overcome some of the shortcomings of conventional cytogenetic analyses. Using recombinant DNA libraries established from sorted human chromosomes, this procedure has made possible the specific delineation ("painting") of individual human chromosomes. In particular, translocations derived from painted chromosomes can be easily recognized [4, 6–8]. The sensitivity of this approach, however, may be limited by the size of the translo-

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Received September 7, 1990; accepted October 8, 1990.

Cancer Genet Cytogenet 57:103–107 (1991)
0165-4608/91/$03.50

cated part of a painted chromosome, depending on the localization of the breakpoint. To study this question and further demonstrate the potential of CISS hybridization for tumor cytogenetics, we selected t(8;21), a nonrandom chromosomal anomaly in ANLL. In this case the translocated part of chromosome 8 is relatively large, whereas the terminal region of 21q reciprocally translocated to the derivative chromosome 8q− is small, frequently escaping detection in conventional banding studies.

MATERIALS AND METHODS

Patients and Cytogenetic Methods

Two female patients aged 59 years (patient 1) and 21 years (patient 2) were diagnosed with typical ANLL-M2 in March and May 1989, respectively. Both patients were alive and in continuing complete remission at the time of this study. At the time of diagnosis, conventional G-banded karyotypes were established after short-term treatment with trypsin, and evaluated according to the ISCN nomenclature [9].

DNA Libraries and CISS Hybridization

Bacteriophage DNA libraries from sorted human chromosomes 8 and 21 were obtained from the American type culture collection (#8: LL08NS02; #21: LA21NS01). Amplification of these libraries, isolation of the bacteriophage DNA, chemical modification by nicktranslation with biotin-11-dUTP, and CISS hybridization were carried out as described in detail elsewhere [5]. Biotinylated DNA sequences were detected by using fluoresceine-isothiocyanate (FITC)-conjugated avidin (green fluorescence). For signal amplification, the protocol of Pinkel et al. [10] was used. Metaphase chromosomes were counterstained with propidium iodide (PI, red fluorescence) and 4,6-Diamidino-2-phenylindol-dihydrochlorid (DAPI, blue fluorescence) and examined with a Zeiss photo microscope III equipped with epifluorescence. Pictures were taken at high numerical aperture with Agfachrome 1000 ASA diapositive films.

RESULTS

G-banding of leukemic cells at the time of diagnosis revealed t(8;21) in all metaphases of patients 1 and 2 with breakpoints in 8q22 and 21q22. It was not clear, however, from these analyses whether the terminal band of chromosome 21 was lost or whether the translocation was reciprocal. In addition, patient 1 showed an interstitial deletion 8q−, whereas both patients lacked one X chromosome in about 25% of the metaphases.

In both cases, CISS hybridization with a chromosome 8 DNA library revealed the translocation of chromosome 8 material to a small acrocentric chromosome (Fig. 1a), which was further identified as chromosome 21 by fluorescence banding with DAPI (not shown). The reciprocal nature of the translocation was demonstrated by CISS hybridization with a chromosome 21 DNA library, which clearly delineated a distinct double spot of yellow-green fluorescent chromosome 21 material on the long arm of the derivative chromosome 8q− (Fig. 1b).

DISCUSSION

In this study, we show that painting of chromosomes 8 and 21 provides a reliable tool for the specific delineation of reciprocal t(8;21)(q22;q22) in two cases of ANLL. In particular, the terminal chromatid material of chromosome 21 translocated recipro-

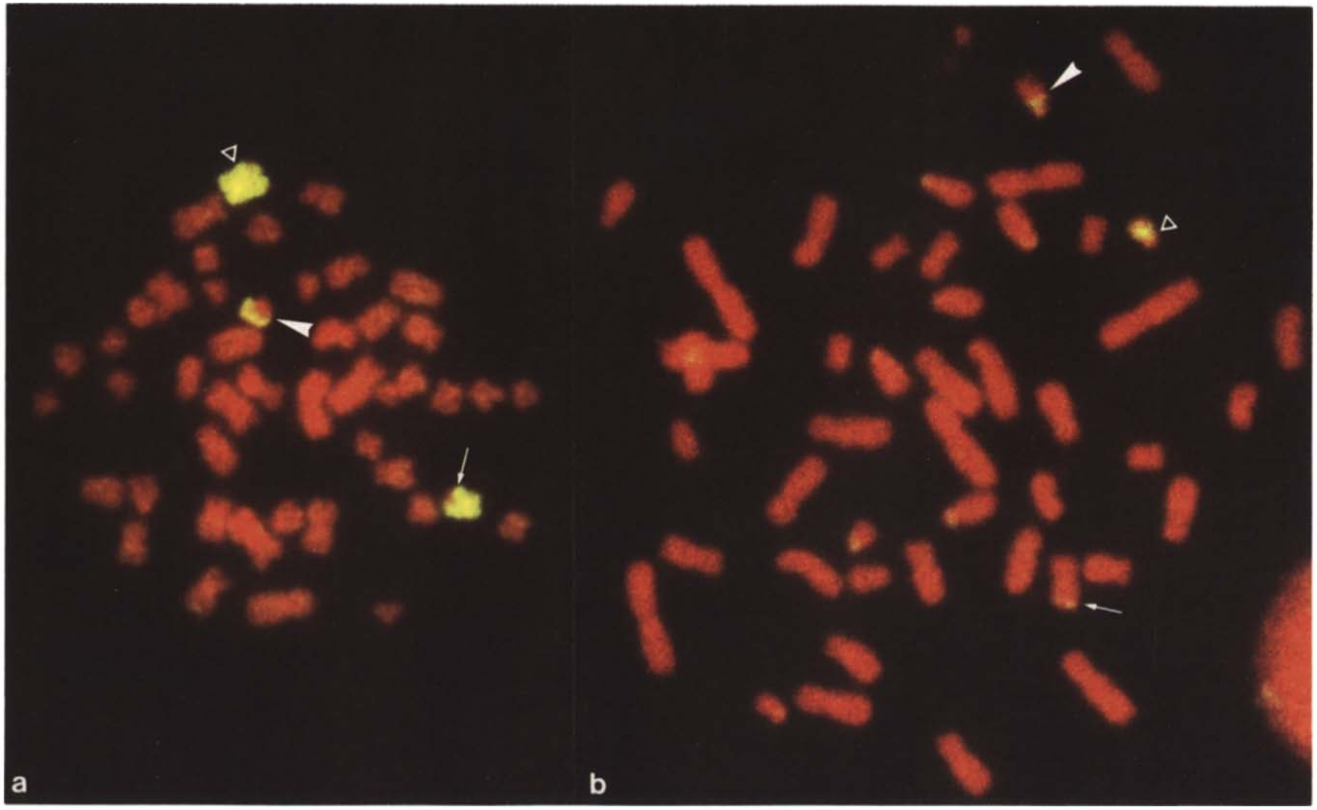

Figure 1 (a) Peripheral lymphocyte metaphase spread from patient 1 after CISS hybridization with a bacteriophage DNA library of chromosome 8. Chromosomes are counterstained with propidium iodide (red fluorescence). Yellow-green fluorescence indicates painted chromosome 8 material, including the normal chromosome 8 painted from pter to qter (open triangle), the derivative chromosome 8q − with a nonpainted, propidium iodide-stained terminal band on the long arm (arrow; compare to Fig. 1b) and the derivative chromosome 21 with translocated chromosome 8 material (arrowhead). (b) Metaphase chromosomes of bone marrow from patient 2 after CISS hybridization with a bacteriophage DNA library of chromosome 21. Painting of chromosome 21 material includes one normal chromosome 21 (open triangle), the derivative chromosome 21 (arrowhead) with yellow-green fluorescent chromosome 21 material and red fluorescent translocated chromosome 8 material, and a small painted region on the derivative chromosome 8q − , indicating the reciprocal translocation of the terminal end of the long arm of chromosome 21 (arrow). Cross-hybridization of chromosome 21 library sequences is indicated by painting of the centromeric heterochromatin/short arm of other acrocentric chromosomes.

cally to the derivative chromosome 8q− was unequivocally detected. This material mostly escapes detection after G-banding analysis. Whereas R-banding may be used to clarify the reciprocal nature of some t(8;21) cases, identification of the translocation depends on sufficiently elongated chromosomes and does not allow a specific identification. In contrast, chromosome painting may be used for the unequivocal identification even in metaphases with rather condensed chromosomes. Accordingly, CISS hybridization with DNA libraries from sorted chromosomes may be recommended as a proper tool for detecting translocations of a few DNA megabases in cases where chromosome banding patterns do not allow a distinctive differential diagnosis between deletion or translocation [8]. Painting of whole chromosomes using DNA libraries from sorted chromosomes provides the advantage that translocations involving any material from the painted chromosome can be detected. CISS hybridization of appropriate cosmid or YAC clones may be used for the identification of still smaller translocations, deletions, or inversions, as well as for the precise definition of breakpoints [11–19].

Part of this work was supported by grants of the Deutsche Forschungsgemeinschaft given to R. B. (SFB 102, project A10, Essen) and T. C. (Cr59/10-1).

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