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Spectral Karyotypic Study of the HL-60 Cell Line: Detection of Complex Rearrangements Involving Chromosomes 5, 7, and 16 and Delineation of Critical Region of Deletion on 5q31.1

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ABSTRACT: *Interstitial deletions of the q arm of chromosome 5 have been associated with acute myelogenous leukemia (AML); therefore, accurate identification of rearrangements of this chromosome in a model cell line, HL-60, is important for understanding the critical genes involved in this disease. In this study, we employed a newly developed technology termed spectral karyotyping to delineate chromosomal rearrangements in this cell line. Our study revealed a derivative of chromosome 7 that resulted from translocations of chromosome arms 5q and 16q to 7q; that is, der(7)t(5;7)(?;q?)t(5;16)(?;q?). Interestingly, both chromosomes 5 and 7 were also involved in translocations with chromosome 16 in der(16)t(5;16)(q?;q?22∼24) and der(16)t(7;16)(?;q?22∼24), respectively. Other notable chromosomal abnormalities that were not previously reported in the HL-60 included an insertion of chromosome 8 in the q arm of chromosome 11, a translocation between chromosomes 9 and 14, and a translocation between chromosomes 14 and 15. In an attempt to define the loss of the 5q31.1 region in HL-60, we performed fluorescence in situ hybridization analysis by utilizing bacterial artificial chromosomes BAC1 and BAC2 that spanned the* IL9 *and* EGR1 *gene interval, which was previously shown to be a critical region of loss in AML. We showed that a copy of both BAC1 (spanning the D5S399 locus) and BAC2 (spanning the D5S393 locus centromeric to BAC1) were present in the normal chromosome 5, but a second copy of BAC1 was lost and a second copy of BAC2 was inserted in the der(16)t(7;16) chromosome. Thus, not only was this study the first to use the new 24-color karyotyping technique to identify several novel chromosomal rearrangements in HL-60, but it also narrowed the 5q31.1 critical region of deletion to the region represented by BAC1.*

INTRODUCTION

The HL-60 cell line was derived from the bone marrow cells of a patient with acute myelogenous leukemia (AML) [1, 2]. This cell line retains many chromosomal abnormalities that were present in the original bone marrow and therefore has become a valuable resource for studying genetic alterations that are associated with development of myeloid leukemias. One of the most consistent chromosomal changes that were present in both the original bone marrow and the HL-60 cells was the loss and rearrangements involving chromosome 5. Deletions of 5q are frequently associated with AML and myelodysplastic syndrome (MDS) [3, 4]. Many investigators have used this cell line to delineate the rearrangement of genes on 5q in an effort to define the critical regions of gene deletion that may harbor tumor-suppressor genes in AML and MDS [5–8].

Previous studies of HL-60 revealed two translocations involving chromosome 5; that is, dic(5;17)(q11;p11) and der(16)t(5;16)(q31;q24) [7, 9]. We recently demonstrated that chromosome 5 sequences were translocated to three

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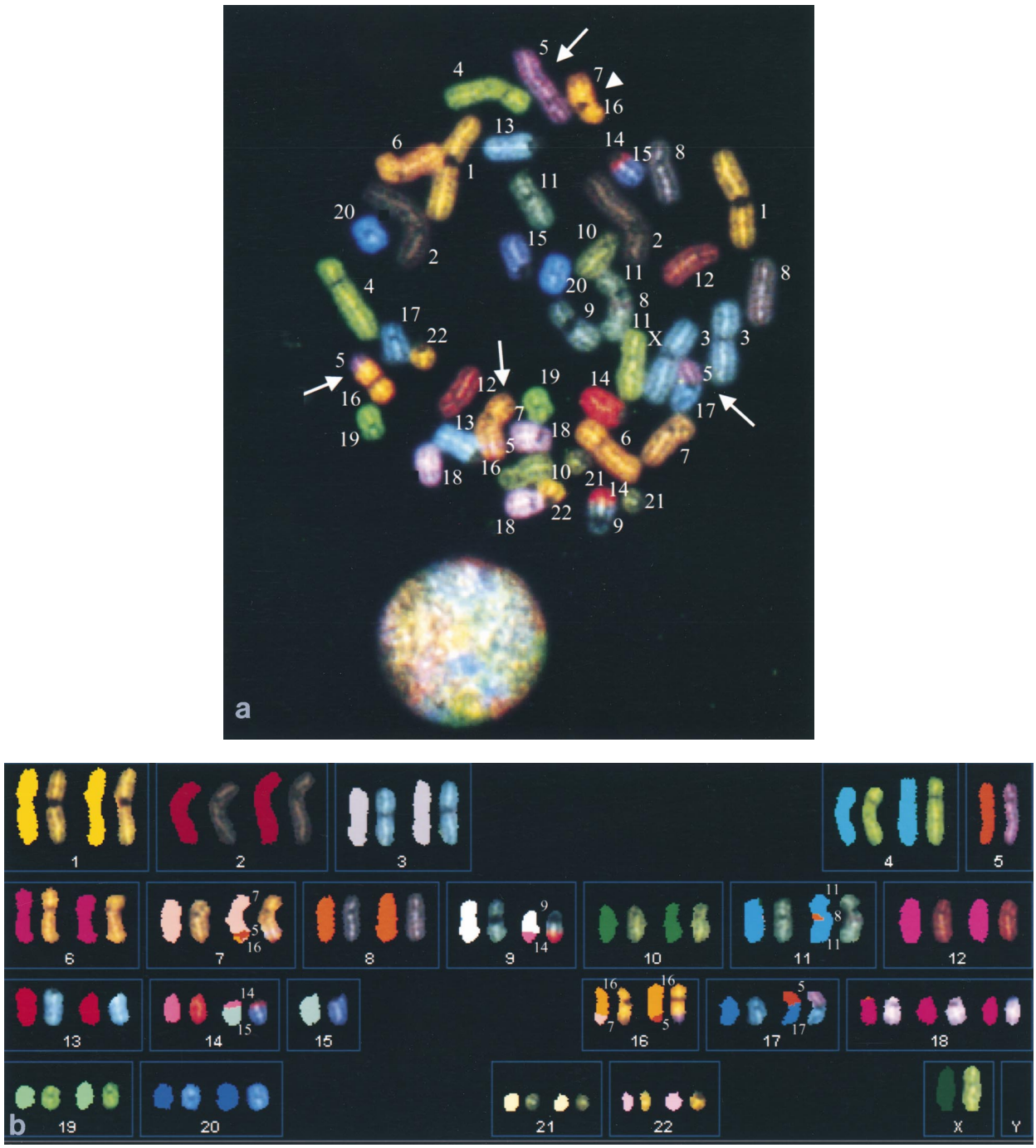

Figure 1 Spectral karyotyping of a metaphase from the HL-60 cell line. (a) Chromosomes were shown in display colors after simultaneous hybridization of 24 combinatorially labeled chromosome painting probes as described by Schröck et al. [10]. Normal chromosome 5 and rearranged chromosomes der(16)t(5;16), der(7), and dic(5;17) that contain chromosome 5 sequences are shown by arrows. An arrowhead shows the der(16)t(7;16) chromosome. (b) Each chromosome was shown in both display and classification colors.

different chromosomes; by using chromosome-specific painting probes, two of which were confirmed to be derivatives of chromosomes 17 and 16, whereas the third chromosome remained unidentified (unpublished). To determine the origin of this unknown translocated chromosome, we employed spectral karyotyping (SKY) [10] and fluorescence in situ hybridization (FISH) methods that utilized both the chromosome-specific painting probes and the chromosome-arm-specific subtelomeric probes [11] to delineate the composition of this rearranged chromosome.

MATERIALS AND METHODS

HL-60 cells at passage 20 were obtained from the American Type Culture Collection (Rockville, MD). Cells at passage 22 were harvested and chromosome preparations were made by conventional cytogenetic methods [12]. FISH was performed by using painting probes and arm-specific subtelomeric probes according to the procedures described by Ning and colleagues, NIH-IMM collaboration [11]. SKY was performed according to the procedures described by Schröck et al. [10]. This method allows all 24 human chromosomes in a cell to be individually color coded after a single FISH procedure.

RESULTS AND DISCUSSION

Figure 1a shows a representative SKY metaphase, from five metaphases analyzed, in which two previously described translocations involving chromosome 5 as identified by arrows can be clearly seen; that is, t(5;17) and der(16)t(5;16) [7, 9]. Also seen in Figure 1a is an insertion in the q arm of chromosome 7. This abnormality had not been reported previously. The inserted sequences could be shown in classification colors in Figure 1b to derive from chromosomes 5 and 16. Subsequent studies by FISH with chromosome painting probes that were specific for chromosomes 5, 7, and 16 confirmed the presence of chromosome 5 and 16 sequences in the q arm of chromosome 7. Using chromosome 7 arm-specific subtelomeric probes (according to the procedures described by Ning and colleagues, NIH-IMM collaboration [11], we also confirmed that the terminal end of the q arm of the der(7) chromosome contained the subtelomeric sequences for 16q, not 7q. Therefore, this newly identified translocation in HL-60 could be described as der(7)t(5;7)(?;q?31)t(5;16)(?;q?). Interestingly, both chromosomes 5 and 7 were also involved in translocations with chromosome 16 in the der(16)t(5;16)(q?;q?22∼24) and the der(16)t(7;16)(?;q?22∼24) chromosomes, respectively (Fig. 1b).

Other notable chromosomal abnormalities that were not previously reported in the HL-60 included an insertion of chromosome 8 in the q arm of chromosome 11, a translocation between chromosomes 9 and 14, and a translocation between chromosomes 14 and 15 (Fig. 1). After determining the modal chromosome number to be 44 in 100 metaphases analyzed and after verifying all chromosomal

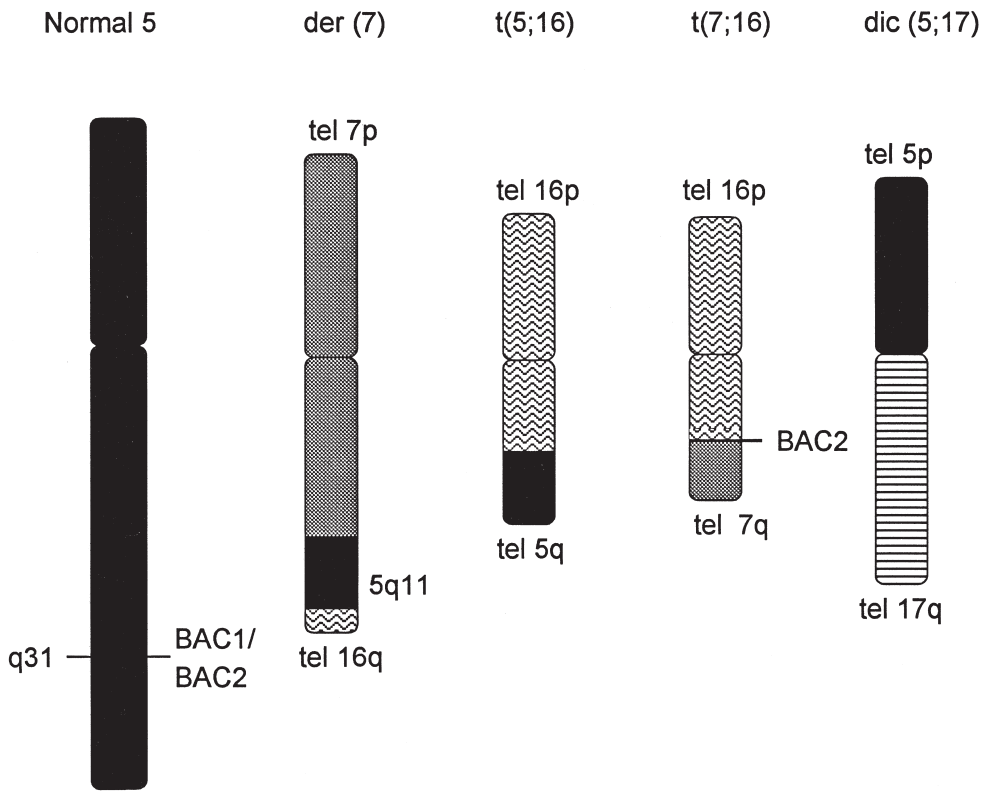

Figure 2 Diagram illustrating rearrangements involving chromosome 5 in HL-60 cells. The der(16)t(5;16) chromosome contained subtelomeric sequences for 16p and 5q at the end of p and q arms, respectively. The der(16)t(7;16) chromosome contained subtelomeric sequences for 16p and 7q. The t(5;17) chromosome contained 5p and 17q subtelomeric sequences. Finally, the der(7)t(7;5)t(5;16) chromosome contained subtelomeric sequences for 7p and 16q at the end of p and q arms, respectively. Also shown in this diagram were the locations of two bacterial artificial chromosomes, BAC1 and BAC2, localized to the critical 5q31.1 region spanning the *IL9-EGR1* interval.

rearrangements observed in SKY with FISH by utilizing chromosome painting and subtelomeric probes, we concluded that the composite karyotype of HL-60 was 44,X, −X,−5,dic(5;17)(q11;p11),del(7)(p?),der(7)t(5;7)(q11;q?31) t(5;16)(q11;q?),add(8)(q?),der(9)del(9)(p2?)t(9;14)(q2?;q2?), del(10)(p?),ins(11;8)(q13?;?),der(14)t(14;15)(q1?;q?),−15, der(16)t(5;16)(q?;q?22∼24),der(16)t(7;16)(?;q?22∼24),+18. This karyotype compared well but showed some discrepancies with that reported earlier by Shipley et al. [7] who used comparative genomic hybridization and FISH methods to determine the karyotype as 46,X,−X,dic(5;17)(q11; p11),hsr(8)(q24),del(9)(p21p23),del(9)(q32),del(10)(p12p15), del(14)(q11),add(16)(q?),der(16)(5;16)(q31;q24),+18,+der (?) t(?;14)(?;q24). Some discrepancies such as modal chromosome number may be due to differences in cell passage used for study. For example, we used an early passage of HL-60 (passage 20), whereas Shipley and co-workers used cells that had been cultured for several years. Other discrepancies such as the del(9)(p21p23), del(14)(q11), and der(?)t(?;14)(?;q24) that were found in Shipley's study probably corresponded to the der(9)del(9)(p2?)t(9;14)(q2?;q2?) and der(14)t(14;15)(q1?;q?) chromosomes in our study. Similarly, the add(16)(q?) chromosome observed in their study most likely was the der(16)t(7;16)(?;q?22∼24) chromosome observed in this study. Furthermore, the add (8)(q?) chromosome observed in our study most likely corresponded to the hsr(8)(q24) in their study.

Because the 5q31.1 region was thought to contain a tumor-suppressor gene that was critical for leukemogenesis of AML, we also performed FISH with bacterial artificial chromosomes (BAC1 and BAC2) that were known to localize in the critical 5q31.1 region spanning the *IL9* and *EGR1* genes [13]. Our results showed that a copy of both BAC1 (spanning the D5S399 locus) and BAC2 (spanning D5S393 locus centromeric to BAC1) were present in the normal chromosome 5, but a second copy of BAC1 was lost and a second copy of BAC2 was inserted in the der(16)t(7;16) chromosome (Fig 1a, indicated by an arrowhead). Further studies showed retention of two alleles of the polymorphic marker D5S526 from BAC2 (Nagarajan, unpublished). Thus, sequences from BAC2 were retained in two copies, whereas flanking loci within the *IL9-EGR1* interval were deleted. Therefore, our study narrowed the area of 5q31.1 deletion in HL-60 to the region represented by BAC1.

Figure 2 illustrates various translocations associated with chromosome 5 in HL-60 cells. One copy of normal chromosome 5 was present on which BAC1 and BAC2 were localized. Sequences from the second copy of chromosome 5 were shown to translocate to three different chromosomes: der (7), t(5;16), and dic(5;17). The p arm of chromosome 5 was previously known to translocate to the dic(5;17) chromosome. The sequences of chromosome 5 in the der(7) chromosome were found to contain sequences from the 5q11 region by mapping studies. The q arm of the der(7) chromosome was shown to have subtelomeric sequences from 16q. The regional derivation of the chromosome 5 sequences on the t(5;16) chromosome were not characterized. Interestingly, mapping studies showed that BAC2 from the 5q31 region was localized near the translocation breakpoint of the t(7;16) chromosome.

In summary, our study identified several chromosomal abnormalities in the early passage of HL-60 cells that were not reported previously. These included der(7) t(5;7)(q?;q?31)t(5;16)(q?;q?), der(9)del(9)(p2?)t(9;14)(q2?;q2?), ins(11;8)(q13?;?), der(14)t(14;15)(q1?;q?), and der(16)t(7; 16)(?;q?22∼24). This study was also the first to identify complex rearrangements involving chromosomes 5, 7, and 16 in the HL-60 cells. Particularly interesting was the finding that a segment of chromosome 5q31.1 sequences was inserted at the translocation breakpoint of the der(16) t(7;16) chromosome. Although these three chromosomes have been known to be aberrant in some AML cells, juxtaposition of sequences from these chromosomes as demonstrated in this study has not been previously reported. This finding may shed some light on the possible interaction or fusion of genes from these three chromosomes that may be important in the leukemogenesis of AML. Finally, our finding of the retention of BAC2 allowed us to exclude some of the genes located within BAC2 as candidate tumor-suppressor genes in AML.

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