DOUBLE-MINUTE CHROMATIN BODIES IN HL-60 LEUKEMIA CELLS SENSITIVE
AND RESISTANT TO DIFFERENTIATION INDUCING AGENTS

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ABSTRACT

We studied the chromosome characteristics of HL-60 promyelocytic leukemia cells sensitive and resistant to differentiation inducing agents (DI). The karyotypic analysis of sensitive (HL-60 S) and resistant (HL-60 R) cells revealed the presence of identical chromosome abnormalities such as loss of chromosomes 5, 9, 10, 14, 16, 17 and X; and gain of chromosome 18. HL-60 S and HL-60 R cells also share five common markers. The difference between the two cell lines consisted essentially of the loss of an unidentifiable chromosome segment in the HL-60 R cell line. In addition, the two sublines showed marked differences in the content of double-minute chromatin bodies (DM), which were abundant in HL-60 S but rarely found in HL-60 R cells. Contrary to a previous report by others, there was no evidence of chromosome rearrangement of the DM as homogeneously stained regions (HSR) or abnormally banding regions (ABR) in the resistant HL-60 R cells. The presence of DM as an expression of gene amplification may be of relevance in the determination of susceptibility of HL-60 cells to DI.

INTRODUCTION

Recently, there have been reports suggesting that the susceptibility of HL-60 cells to DI is associated with certain chromosomal abnormalities. The presence of DM in metaphase preparations of HL-60 has been demonstrated in only those cells sensitive to DI (Au et al., 1983). Moreover, the c-myc gene was shown to be amplified in DM of sensitive HL-60 cells (Collins et al., 1982).

In our laboratory, we were able to characterize two sublines of HL-60 cells which differed markedly in their ability to undergo differentiation to granulocytes after treatment with various DI. HL-60 S cells were sensitive to retinoic acid, dimethyl sulfoxide and other DI as previously reported (Collins et al., 1980). However, HL-60 R cells were highly resistant to the same agents (Leyva et al., 1986). Resistance to DI was completely reversed in HL-60 R cells by combined treatment with retinoic acid, dime-

thyl sulfoxide and the DNA-hypomethylating agent, 5-aza-2'-deoxycytidine (Schwartsmann et al., 1987).

In this paper, we report on the cytogenetic features of HL-60 S and HL-60 R cells, aiming to provide additional information on the chromosomal characteristics of HL-60 cells associated with susceptibility to induction of differentiation.

MATERIAL AND METHODS

Cell culture

Original HL-60 cells were provided by Drs. S.J. Collins and R.C. Gallo (NCI, Bethesda, USA) and designated HL-60 S. An HL-60 subline that was unresponsive to DI was obtained from Dr. L. Smets (Netherlands Cancer Institute, Amsterdam, The Netherlands) and designated HL-60 R. The latter subline had developed spontaneously after numerous passages under ordinary culture conditions. Examination of cells stained with May-Grunwald-Giemsa showed that both HL-60 S and HL-60 R were morphologically similar, comprising more than 90% promyelocytes.

Cells were propagated as stationary-suspension cultures in RPMI-1640 medium containing 25 mM HEPES (Serva, Heidelberg, FRG) and 10% heat-inactivated fetal bovine serum (Flow Labs., Zwanenburg, The Netherlands) at 37°C in 5% CO₂ - 95% air atmosphere. Cells were passaged twice weekly at an initial concentration of 3×10^5 cells/ml. Experiments were performed with cells in logarithmic growth. HL-60 S cells grew with a doubling time of 32 hr and HL-60 R grew with a doubling time of 39 hr.

Cytogenetics

Chromosome preparations were made using standard techniques from exponentially growing cultures of HL-60 cells. The chromosomes were G-banded using the trypsin-Giemsa (Seabright, 1971) and the high-resolution banding methods (Yunis, 1976).

RESULTS

In HL-60 S, chromosomes were counted in 30 cells without banding and 24 cells were analyzed in detail in G-banded slides. In HL-60 R, chromosomes were counted in 24 cells and 7 cells were analysed in G-banded slides. In HL-60 S, the majority of cells (19/30) had 45 chromosomes while cells (9) with 46 chromosomes were also present. In cells with 46 chromosomes, the extra chromosome was either a very small acrocentric or a ring chromosome smaller than a 21. The modal number of chromosomes in HL-60 R cells was 44.

The HL-60 S subline showed loss of chromosomes 5, 9, 10, 14, 17, both 16 s and X and a gain of 18. There were six marker chromosomes present: M1-M6 (see table 1). In HL-60 R the same chromosome constitution was found, except that three chromosomes were missing, namely: 6, 21 and M6. These were replaced by M7 and M8. M7 appears to consist of the long arm of M6 (10q) and the long arm of 6 (6q). M8 consists of the short arm of 6 (6p), attached to the long arm of 21 (21q), i.e. essentially the whole of chromosome 21. DM were present in all 24 banded HL-60 S cells, ranging from two to 26, with an average of nine per cell. In contrast, DM were absent in all except one of the HL-60 R cells. The main chromosome abnormalities found in HL-60 S and HL-60 R cells are summarized in Table 1.

TABLE 1. CHROMOSOME ABNORMALITIES IN HL-60 CELLS

Characteristic	HL-60 S	HL-60 R
Modal Chromosome Number	45	44
Double-Minutes (DM)	abundant	rare
Chromosome Losses	-5	-5
	-9	-6
	-10	-9
	-14	-10
	-16	-14
	-16	-16
	-17	-16
	-X	-17
		-21
Chromosome Gains	+18	-X
Markers (karyotypic description)		+18
M1 (9p-)	+	+
M2 (16q+)	+	+
M3 (16q++)	+	+
M4 (17p+)	+	+
M5 (inv(14)(q24q32))	+	+
M6 (10p+)	+	-
M7 (t(10q;6q?))	-	+
M8 (t(6p;21q))	-	+

p= short arm, q= long arm (+ or ++ are used to indicate the degree of increase in length); marker chromosomes are described as present (+) or absent (-).

DISCUSSION

DM, HSR and ABR probably represent alternative forms of gene amplification (Alitalo et al., 1983). The presence of DM in HL-60 cells appears to be associated with sensitivity to DI (Au et al., 1983). Perhaps, the amplification of certain genes such as c-myc in the DM could be related to the susceptibility of HL-60 cells to undergo differentiation after exposure to DI (Collins et al., 1982).

In our study, the karyotypes of the two sublines of HL-60 revealed the same gains and losses of nine chromosomes and five common markers. However, DM were abundant in the sensitive HL-60 S cells but rare in the resistant HL-60 R cells, consistent with the above mentioned previous reports. Nevertheless, no HSR or ABR was found in HL-60 R cells, that could account for a redistribution of presumably amplified sequences in DM. The appearance of HSR or ABR after the loss of DM have been previously reported in other cell lines from neuroblastoma and adrenocortical tumor (George et al., 1980). In HL-60 cells an ABR in chromosome 8 was found after long-term culture and the disappearance of DM (Nowell et al., 1983). HL-60 R we describe here appears to be a distinctly different type of HL-60 variant resistant to induction of differentiation.

Apart from the DM content, other karyotype differences were found between HL-60 S and HL-60 R cells. The complex translocation in HL-60 R cells, involving chromosomes M6, 6 and 21 accounts for the modal number of 44 in this subline, as compared to 45 chromosomes in HL-60 S cells. The banding patterns of M7 and M8 in HL-60 R correspond to that of chromosomes 6, 21 and the long arm of M6 in HL-60 S. This suggests that the only difference between the two karyotypes is the loss of the short arm of M6 in resistant cells. The banding pattern of this chromosome segment is not distinct and its origin cannot be identified.

At present, we are using in situ hybridization techniques to further characterize these chromosome rearrangements in HL-60 cells. Of particular interest is the localization of certain genes such as c-myc in sensitive and resistant cells, to determine whether such amplified sequences remain localized, distribute more widely or diminish with the loss of DM. Such studies could have important implications for explaining susceptibility and resistance to DI.

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