GENOTYPIC AND PHENOTYPIC CHANGES DETERMINING RESISTANCE OF DJUNGARIAN HAMSTER CELLS TO ADRIABLASTIN

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Resistance of tumor cells to the antitumor agent adriablastin (synonym: adriamycin) arises in some cases as a result of a decrease in accumulation of the drug by resistant cells [10, 11]. Cells resistant to adriablastin acquire cross-resistance to a number of agents differing in their chemical structure and mechanism of action: actinomycin D, vincristine, daunomycin, etc. [10, 11]. This suggests that resistance to adriablastin may be based on changes in permeability of the plasma membrane for the cytostatic [10, 11]. A decrease in permeability of the plasma membrane also determines resistance to colchicine [8, 9, 13]. Cells resistant to colchicine, incidentally, possess cross-resistance of adriablastin and to preparations to which adriablastin-resistant variants are cross-resistant [8, 13]. The writers showed previously that resistance of cells to colchicine is connected with the amplification gene [2-6, 12]. The development of resistance to colchicine in Djungarian hamster cells is accompanied by a regular evolution of the karyotype: the appearance of an extra chromosome 4, the appearance of small chromatin bodies (SCB), the formation of a long, homogeneously stained region (HSR) which, like SCB, is a structure containing amplified genes, in one of the three chromosomes 4, and also by transposition of HSR to other chromosomes of the set, and so on [2-6, 12].

In this investigation an attempt was made, first, to discover whether the onset of resistance to adriablastin in Djungarian hamster cells is accompanied by the appearance of cytologic features of gene amplification (HSR in chromosomes, SCB, double microchromosomes): second, to compare changes in karytotype taking place during the development of resistance to colchicine and adriablastin, and third, to compare changes in permeability of the plasma membrane in cells selected with the aid of colchicine and adriablastin.

EXPERIMENTAL METHOD

Djungarian hamster cells of line DM-15 [1, 2] and cells of sublines $DM^{5/1}$, $DM^{5/5}$, $DM^{5/7}$, and $DM^{5/10}$ obtained from them, which are 750-800 times more resistant to colchicine than the wild-type cells [4, 6].

Cells resistant to adriablastin were obtained from DM-15 cells by multistage selection. In the first stage of selection cells resistant to 0.025 μg/ml adriablastin (from Farmitalia, Italy) were isolated. Colonies of these cells appeared with a frequency of about 10^{-4}. The isolated cells, designated $DM^{adb-0.025}$, were at least 8 times more resistant to the selective agent than the wild-type cells (LD_{50} of adriablastin for the original DM-15 cells was 0.0030-0.0035 μg/ml). By means of selection, in medium with increasing concentrations of adriablastin (at each stage of selection the dose of the drug was increased by 2-2.5 times) a number of sublines were obtained, two of which ($DM^{adb-0.2}$ and $DM^{adb-0.8}$) were resistant to 0.2 μg/ml (degree of resistance ≥ 70) and 0.8 μg/ml (degree of resistance ≥ 270) of adriablastin, were used, together with $DM^{adb-0.025}$ cells, in the present investigation.

The conditions of culture of the cells, the technique of obtaining the chromosome preparations, G-band staining and analysis, and the methods of studying accumulation of [^{3}H]-

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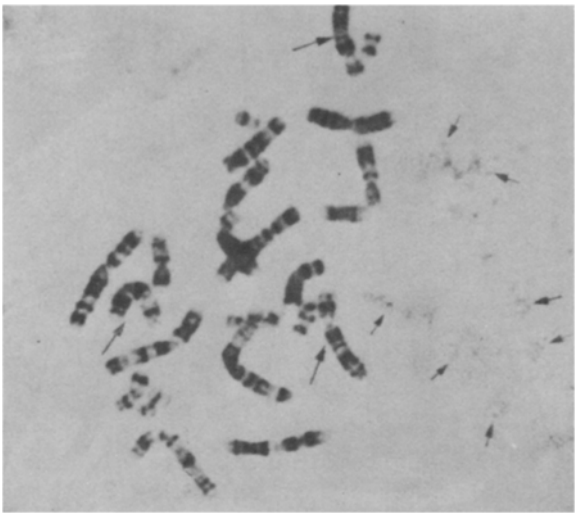

Fig. 1. Metaphase plae of a $DM^{adb-0.2}$ cell. G-band staining. 28 chromosomes, trisomy for chromosome 4 (large arrows), clusters of SCB (small arrows).

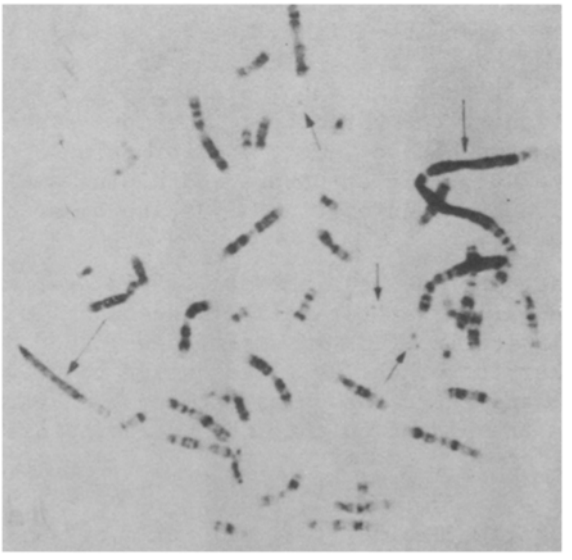

Fig. 2. Metaphase plate of a $DM^{adb-0.8}$ cell. G-band staining. 47 chromosomes, 2 marker chromosomes of uncertain origin with long HSR (large arrows), SCB (small arrows).

colchicine, [^{3}H]puromycin, [^{3}H]actinomycin D, [^{3}H]cytochalasin B, and [^{3}H]vinblastine (all from Amersham Corporation, England), were described by the writers previously [2-7, 14].

EXPERIMENTAL RESULTS

Analysis of the chromosome preparations after G-band staining showed that changes in karyotype observed in DM-15 cells in the course of development of resistance of adriablastin were very similar to the changes of karyotype described by the writers previously [2-6, 12] which accompany the development of resistance to colchicine. In both cases, an extra (a third) chromosome 4 was found in cells selected at the first stage (degree of drug resistance 8-20). In subline $DM^{adb-0.025}$ the fraction of cells with trisomy for chromosome 4 (Fig. 1) was 95%. In 5% of cells of this subline, besides the extra chromosome 4, there were single SCB — structures described for the first time in colchicine-resistant cells, and containing amplified genes [5, 6].

To raise the level of drug resistance the number of SCB in the cells and the fraction of metaphases containing these formations were increased. For instance, in $DM^{adb-0.2}$ cells,

TABLE 1. Accumulation of Some Preparations by Colchine-Resistant Cells $DM^{5/1}$ and $DM^{5/5}$, Adriablastin-Cells $DM^{adb-0.8}$, and Wild-Type DM-15 Cells

Compound	dpm 10^{-3}			
	$DM^{5/1}$	$DM^{5/5}$	$DM^{adb-0,8}$	DM-15
[^{3}H]colchicine (0.4 μCi/ml)	2,9±0,1	2,6±0,3 c	2,7±0,1	14,4±0,7
[^{3}H]actinomycin D (0.4 μCi/ml)	15,7±0,3	32,9±1,7	35,9±0,5	102,9±1,9
[^{3}H]puromycin (0.4 μCi/ml)	5,9±1,3	7,4±0,3	14,2±1,4	50,0±1,1
[^{3}H]cytochalasin B(0.2 μCi/ml)	0,9±0,1	1,2±0,1	1,3±0,1	25,7±2,1
[^{3}H]vinblastine (0.1 μCi/ml)	1,3±0,3	1,7±0,1	2,1±0,1	25,1±0,2

Legend. Cells of all sublines were seeded at the rate of 10^6 per flask with an area of bottom of 20 cm^2. Next day the cells were incubated for 4 h with the labeled compounds, washed to remove extracellular label, dissolved, and the amount of radioactivity was determined in the samples [7, 8, 14].

approximately 70 times more resistant to adriablastin, 70% of the cells contained larger accumulations of SCB (Fig. 1). All cells of the given subline preserved the extra chromosome (Fig. 1). In 7.5% of $DM^{adb-0.2}$ cells, an HSR of small size was found in one of the three chromosomes 4.

A further increase in resistance to adriablastin was accompanied by the appearance of hypotetraploid variants containing markers of uncertain origin with long HSR. The karyotype of all cells of subline $DM^{adb-0.8}$ (degree of resistance ≥ 270) contained 46-48 chromosomes. Many new markers appeared in it, including 1-3 chromosomes with long HSR (Fig. 2). The fraction of cells in which long HSR were found was 64% in subline $DM^{adb-0.8}$. In addition, about 30% of cells contained accumulations of SCB; in 7.5% there were both SCB and chromosomes with long HSR (Fig. 2).

The development of resistance of adriablastin in the population of Djungarian hamster cells of line DM-15 is thus accompanied by the appearance of cytologic features of gene amplification: SCB and HSR of the chromosomes. Under these circumstances, the order of changes in karyotype in the cell system studied (the appearance of an extra chromosome 4, the appearance of SCB, formation of HSR in one of the chromosomes 4, transposition of HSR to other chromosomes of the set), observed in the course of development of resistance to adriablastin, was identical with the evolution of the karyotype described by the writers previously (2-6, 12] during the development of resistance to colchicine. In this connection it seemed very probable that resistance to adriablastin arises as a result of amplification of the same gene whose multiplication leads to the formation of resistance to colchicine.

Evidence in support of this hypothesis is given by the similarity between the changes in the plasma membrane which was found in colchicine-resistant and adriablastin-resistant variants (Table 1). It will be clear from Table 1, which gives the results of one experiment, that cells of colchicine-resistant sublines $DM^{5/1}$ and $DM^{5/5}$ accumulate much less [^{3}H]colchicine, [^{3}H]actinomycin D, [^{3}H]puromycin, [^{3}H]cytochalasin B, and [^{3}H]vinblastine than the original parental DM-15 cells. A decrease in accumulation of the above compounds also was found in cells of two other colchicine-resistant sublines: $DM^{5/7}$ and $DM^{5/10}$ (data not given in Table 1). Cells $DM^{adb-0.8}$, selected by means of adriablastin, also had reduced permeability of the plasma membrane for all the above compounds. Accumulation of each of the preparations, it will noted, was reduced approximately by the same degree in adriablastin-resistant and colchicine-resistant cells (Table 1).

This investigation thus showed that the development of resistance to adriablastin, like the development of resistance to colchicine, is evidently connected with amplification of the gene (genes) whose increased expression causes a decrease in permeability of the plasma membrane for certain compounds. In the next communication data will be given on identification of a low-molecular-weight protein hyperproduced in colchicine-resistant and adriablastin-resistant cells. Probably resistance to colchicine and adriablastin is based on amplification of the gene (genes) of this protein.

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HYPERPRODUCTION OF A SPECIFIC PROTEIN IN CELLS RESISTANT TO COLCHICINE AND ADRIABLASTIN

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KEY WORDS: colchicine; adriablastin; gene amplification; protein synthesis; permeability of the plasma membrane.

Resistance of mammalian somatic cells to certain cytotoxic agents (methotrexate, N-phosphoacetyl-L-aspartate, etc.) arises as a result of amplification of the genes of proteins whose hyperproduction is responsible for the development of drug resistance [7, 9, 11, 12]. The writers showed previously that resistance of Djungarian hamster and mouse cells to colchicine is connected with gene amplification, leading to a decrease in permeability of the plasma membrane for colchicine and certain other substances [2-4, 6]. It has also been found that approximately the same changes in genotype and plasma membrane permeability take place in cells selected for resistance to adriamycin as in cells isolated in the presence of toxic concentrations of colchicine [4].

In the investigation described below a protein hyperproduced in colchicine-resistant and adriablastin-resistant cells, containing amplified genes, was identified by two-dimensional electrophoresis.

EXPERIMENTAL METHOD

Djungarian hamster cells of line DM-15 [1], colchicine-resistant cells of sublines $DM^{5/1}$ and $DM^{5/5}$ [2, 3], and adriablastin-resistant cells of subline $DM^{adb-0.8}$ [4], obtaining from them, and also mouse cells of lines L and L-53 (a subline of L cells resistant to colchicine [5]), were used.

The conditions of culture of the cells and the degree of their drug resistance were described previously [2-4].

Proteins from sensitive and resistant cells were analyzed by two-dimensional gel-electrophoresis by O'Farrell's method [10] with certain modifications. Cells growing on dishes 60

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