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CROSSED RESISTANCE OF TUMOR CELLS WITH HIGH RESISTANCE TO COLCHICINE

R. I. Abdryashitov and A. A. Stavrovskaya

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During chemotherapy with any antitumor preparation the tumor cells may acquire resistance not only to the same preparation, but also to several other chemical compounds differing in their structure and mechanism of action. This phenomenon has been called multiple drug resistance (MDR). Despite much progress in the study of MDR [1, 2], many aspects of this phenomenon remain unclear. A matter of undoubted interest for the understanding of the mechanisms of MDR is a study of crossed resistance (CR). The list of substances exhibiting CR is long and includes the Vinca alkaloids, colchicine, anthracycline antibiotics, and antinomycin D. Investigation of various cell lines with MDR has led to the following ideas: during the development of MDR the highest level of resistance is observed most frequently to a selective agent, and the level of CR to other preparations is as a rule lower. The degree of CR in different cell lines varies considerably, but it usually correlates directly with the level of resistance to the selected agent [1, 2]. However, this picture is not always observed. For instance, CR to Vinca alkaloids and to gramicidin C in some cell lines with MDR was found to be considerably higher than resistance to the selective preparation [3, 4]. In human cells resistant to podophyllotoxin, CR was observed to a wide range of preparations, but sensitivity to Vinca alkaloids, colchicine, and actinomycin D was completely preserved [5].

In the investigation described below, to study the causes and principles of variability of the character of CR in MDR, a series of cell lines with a very high level of resistance to colchicine was obtained. This model was used to study the development of CR to several preparations while the resistance to the selected agent was increased.

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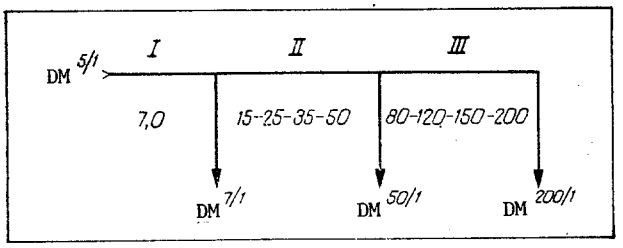

Fig. 1. Diagram showing method of obtaining sublines of Djungarian hamster fibroblasts with a high degree of resistance to colchicine. Numbers indicate increase in colchicine concentrations in medium (in μg/ml).

TABLE 1. Resistance to Colchicine and Other Preparations (CR) in a Series of Sublines of Djungarian Hamster Fibroblasts with an Increasing Degree of Resistance

Name of line	LD_{50}, μg/ml*				Degree of resistance**			
	colchicine	vincristine	actinomycin D	adriablastin	colchicine	vincristine	actinomycin D	adriablastin
DM-15	0,015	0,0075	0,002	0,025	1	1	1	1
$DM^{0.1/1}$	0,16	n.d.	0,008	n.d.	11	—	4	—
$DM^{5/1}$	10,6	5,5	0,67	0,9	707	730	335	36
$DM^{7/1}$	12,0	5,75	0,68	2,4	800	770	340	96
$DM^{50/1}$	66,3	11,5	0,7	2,75	4 420	1530	350	110
$DM^{200/1}$	250,0	20,5	0,72	5,25	16 670	2730	360	210

Legend. *) Dose of preparation reducing number of colonies of cells grown in medium with preparation by 50%, **) ratio of LD_{50} for resistant cells to LD_{50} for DM-15 cells. n.d.) Not determined.

EXPERIMENTAL METHOD

Djungarian hamster fibroblasts of the DM-15 strain [6] and their derivatives, namely sublines $DM^{0.1/1}$ and $DM^{5/1}$, resistant to colchicine [7], were used. All cell lines were cultured on Eagle's medium with the addition of 10% bovine serum and monomycin (100 μg/ml). Lines highly resistant to colchicine were obtained by multistage selection from the original $DM^{5/1}$ line. The process of obtaining highly resistant lines can be conventionally divided into several stages, which differ from each other in by how much the dose of colchicine was increased during each consecutive round of selection (Fig. 1). The cells (5×10^6) were seeded into Petri dishes 100 mm in diameter and colchicine was added to the culture medium during seeding. In the course of selection, death of no fewer than 80% of the seeded cells took place. The culture resistant to a given concentration of the cytostatic was usually formed after 10-15 passages through culture in the presence of colchicine, taking 1.5-2 months. As a result of this long-term selection a number of sublines of common origin was obtained, and identified as $DM^{7/1}$-$DM^{200/1}$, having been selected in medium containing 7-200 μg/ml of colchicine, respectively. The degree of resistance of the sublines thus obtained to colchicine was tested by determining the efficiency of colony formation in medium with different concentrations of the preparations. Besides colchicine (from "Merck," West Germany), the following reagents also were used: actinomycin D ("Reanal," Hungary), adriablastin ("Farmitalia," Italy), vincristine ("Reanal"). The protein spectrum was analyzed by one-way PAG electrophoresis with sodium dodecylsulfate, by Laemmli's method [8].

EXPERIMENTAL RESULTS

A series of cell lines highly resistant to colchicine was obtained by multistage long-term selection (Fig. 1). The lines obtained were 800-16,600 times more resistant to colchicine than the original DM-15 cells (Table 1). Series of cell lines with such a high level of resistance to vegetable alkaloids have not previously been described. The fact that these lines could be obtained is evidence of the great capacity of the system responsible for this type of protection of cells against toxic action. The character of CR of

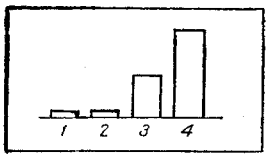

Fig. 2. Relative content of 22 kD protein in sublines of Djungarian hamster fibroblasts. 1) DM-15; 2) $DM^{0.1/1}$; 3) $DM^{50/1}$, 4) $DM^{200/1}$.

Djungarian hamster cells with a level of resistance from 11 to 700 times ($DM^{0.1/1}$-$DM^{5/1}$) did not differ from the most commonly found type of CR. The level of resistance to colchicine exceeded the levels of resistance to other preparations, and with an increase in resistance to colchicine, the level of CR also rose. For instance, when resistance of sublines $DM^{0.1/1}$ and $DM^{5/1}$ to colchicine and antinomycin D was compared, the parameters characterizing resistance of the cells to the two preparations rose by about the same degree, namely by 60-80 times (Table 1).

The study of lines with a high level of resistance to colchicine showed that in some of these lines the change in CR was different in character (Table 1). Whereas resistance to the selective agent in several lines increased considerably (the level of resistance to colchicine of $DM^{200/1}$ cells exceeded the level of resistance of $DM^{7/1}$ by more than 20 times), the degree of resistance to actinomycin D was virtually unchanged. The level of resistance to vincristine and adriablastin rose only a little – by 2-4 times. Thus the character (profile) of CR in lines with a high level of resistance differs from that for lines with lower resistance.

Investigation of the protein spectrum of cell lines with a high level of resistance to colchicine showed that with an increase of resistance to colchicine the content of protein with molecular weight of 22 kD increased (Fig. 2).

MDR is a complex phenomenon. The commonest change in this type of drug resistance is hyperexpression of a 170 kD protein [1, 2]. Experiments with introduction of the gene coding this protein into cells show that an increase in the number of its copies can give rise to typical MDR with characteristic CR [9]. More recently, however, it has become evident that the mechanisms determining MDR are quite varied. For instance, cell lines with MDR have been described in which hyperexpression of p170 is absent, or in which other proteins are hyperexpressed [5, 10]. In human cells of the KB line, selected for resistance to colchicine, mutations have been found at certain sites of an amplified gene coding for p170 [11]. The authors cited connect the characteristic CR profile, namely higher resistance to the selective substance than to other agents, with these secondary genetic changes. With an increase in resistance to colchicine, in the series of cell lines which we investigated, an increase in the content of p22 protein was observed. Small cytosol proteins of this kind with affinity for calcium have been found in several cells with MDR [2, 12]. The role of these proteins in MDR is not clear. Proteins similar to p22 may perhaps somehow affect the working of membrane protein p170. It can be tentatively suggested that these proteins transport toxic substances in the cytoplasm, or influence it in some way or other. In our view, the series of cell lines described in this paper provides an approach to the study of the function of p22 in cells with MDR.

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