L-CELL SUBSTRAIN RESISTANT TO METAPHASE INHIBITORS

A. A. Stavrovskaya

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By continuous cultivation of L-cells in a medium with an increasing concentration of colcemid a subline L-53, 50 times more resistant to the mitostatic action of colcemid than the original L strain, was obtained. The L-53 cells also were resistant to other metaphase inhibitors: vincristin, vinblastin, and 17β-estradiol. The resistant and sensitive sublines were indistinguishable in their chromosomal characteristics.

Comparison of the sensitivity of normal and transformed cell strains to colcemid has shown that most strains which have undergone malignant transformation are more sensitive to the mitostatic action of this metaphase inhibitor than normal cells [2]. Since increased sensitivity to agents damaging the division spindle may be one factor concerned in the production of aneuploidy of tumor cells there is a need for a closer study of the mechanisms determining variation in cell sensitivity to metaphase inhibitors. Substrains of the same strain, differing in their sensitivity to mitostatics, provide a convenient model for such investigations. No such strains capable of growth in culture yet exist.

This paper describes a substrain of the L strain of transplantable mouse fibroblasts, identified by the number L-53, resistant to colcemid.

EXPERIMENTAL METHOD

The following metaphase inhibitors were used: colcemid (Ciba Laboratories Limited), vincristin (Eli Lilly and Co.), vinblastin (Richter), and 17β-estradiol (Calbiochem).

Strain L cells [7] were grown in Carrel's flasks as described previously [1]. The substrain resistant to colcemid was obtained by growing the cells continuously in the presence of colcemid. Seeding was carried out after 3-5 days at the rate of $2 \cdot 10^5$ cells per ml, in a volume of 5 ml per flask. Colcemid was added 1-2 h after seeding. The medium was changed on alternate days, when fresh colcemid was added. A control culture of L-cells, subcultured after the same time intervals as the L-53 strain in the same culture medium, with the same number of cells, was maintained parallel with the experimental culture.

The colcemid concentration in the medium was increased gradually, starting from a dose of 0.005 μg/ml. At the 33rd subculture after the beginning of the experiment the concentration of colcemid in the medium reached 0.2 μg/ml, and it remained at this level during subsequent subcultures.

The sensitivity of the cells to the mitostatic action of the metaphase inhibitors was assessed as follows: Cells of the sensitive and resistant substrains were seeded into penicillin flasks with coverslips on the bottom at the rate of $4 \cdot 10^5$ cells to 2 ml of medium. Substrain L-53 was seeded without colcemid. The sensitivity of the cells to the metaphase inhibitors was determined 24 h later [1]. A decrease in the proportion of postmetaphase stages of mitosis under the influence of increasing doses of the substances pointed to sensitivity of the cells to the dose of the agent producing this decrease. To compare the sensitivity of the L and L-53 cells to colcemid the value of ED_{50} (the dose of the preparation through the action of which the proportion of postmetaphase stages of mitosis was reduced by 50% compared with the control) was calculated by the probit method.

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TABLE 1. Decrease in Proportion of Postmetaphase Stages of Mitosis in Substrains Sensitive (L) and Resistant (L-53) to Colcemid under the Influence of Metaphase Inhibitors

Substrain	Vincristin (μg/ml)				Vinblastin (μg/ml)				Ethanol, 0.5% solution (solv. for estradiol)	17β-estradiol ($\cdot 10^{-4}$ M)	
	Control	0.01	0.1	1.0	10.0	0.001	0.01	0.1	1.0	0.25	1.25	5.0
L	50,4±3,9	16,5±7,3	0,25±0,5	0	—	47,2±1,25	8,5±11,4	0	—	49,0±2,8	24,0±9,4	2,7±0,9	2,7±2,6
L-53	53,0±7,6	49,5±3,5	49,2±11,2	12,5±2,12	0,5±0,7	—	54,0±5,1	52,0±1,5	1,0±0	43,5±3,5	33,5±3,5	20,5±1,3	13,2±8,2

Dried chromosomal preparations were made by the usual method, and this material was analyzed under the microscope by examination of 50 metaphase plates.

EXPERIMENTAL RESULTS

Sensitivity of the L and L-53 cells to colcemid was compared at the 78th subculture from the beginning of the experiment to obtain a resistant substrain. For the L-cells, ED_{50} of colcemid was 0.0064 (0.0059-0.0069) μg/ml, P = 0.05. For the L-53 cells ED_{50} was 0.345 (0.303-0.393) μg/ml, with the same value of P. The value of ED_{50} of colcemid for the L-53 cells was thus more than 50 times greater than ED_{50} for the control substrain, i.e., L-53 cells are 50 times more resistant to colcemid than the original L strain.

The resistant strain has now passed through more than 100 generations with colcemid present continuously in the medium, but its cells still retain a high level of resistance even after prolonged subculture without colcemid. This suggests that resistance to the action of colcemid has become an inherited character of the cells. It is clear from Fig. 1 that during cultivation of strain L-53 without colcemid its sensitivity to the compound increases, but even three months after removing the colcemid it is still below the control level. A decrease, or even disappearance, of the resistance of cells to various substances in the absence of the selective agent has been described previously [6].

Some workers who have studied cells resistant to amethopterin consider that the development of resistance may be connected with changes in the karyotype [4, 5]. To determine whether the appearance of resistance to colcemid is connected with any visible changes in the chromosomes, the karyotypes of the resistant and sensitive substrains were compared. As Fig. 2 shows, substrain L-53 was virtually indistinguishable in its karyotype from the original L strain: the modal class in both substrains was formed by cells with 57-60 chromosomes, and the number of two-arm chromosomes was the same. The two substrains also were identical as regards the character of the markers: in both strains cells were found with a small telocentric chromosome, one small and one or two medium-sized subtelocentrics, and a small metacentric chromosome. On analysis of substrain L-53 at the 49th subculture, in most cells a very large submetacentric marker with a secondary constriction band in the long arm was found. However, on subsequent investigations of strain L-53 no marker subtelocentrics could be found. This chromosome was probably accidental for substrain L-53. Various changes in karyotype can, of course, be observed in cell strains during prolonged subculture. Great care must evidently be exercised when associating visible changes in chromosomes with particular changes in the phenotype of these strains.

During the investigation of chromosome preparations in the first stages of obtaining the strain many polyploids were found: 26% at the 15th subculture. The number of polyploid cells gradually fell to reach the control level (2.8%) by the 56th subculture, subsequently remaining at the same figure.

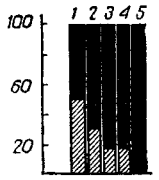

Fig. 1

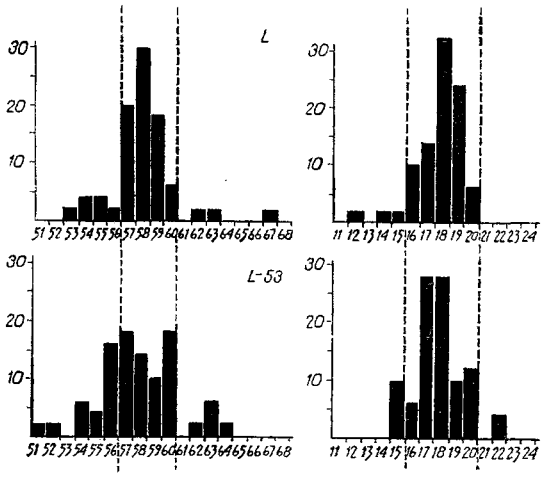

Fig. 2

Fig. 1. Changes in ratio between pre- and postmetaphase stages of mitosis in substrain L-53 grown in the presence of colcemid (in a concentration of 0.05 μg/ml), and the same 2-12 weeks after removal of colcemid. Abscissa: 1) culture of cells of substrain L-53 grown in the presence of colcemid; 2) culture of L-53 cells grown without colcemid for two weeks; 3) the same, grown for six weeks; 4) the same, grown for 12 weeks; 5) original culture of L-cells. Ordinate, percentage of mitotic cells in premetaphase (black columns) and postmetaphase (obliquely shaded columns) stages of mitosis.

Fig. 2. Distribution of cells of substrains L (above) and L-53 (below) by total number of chromosomes and by number of two-arm chromosomes. Abscissa, total number of chromosomes (on left) and number of two-arm chromosomes (on right); ordinate, percentage of cells with the given number of chromosomes.

With the acquisition of resistance to colcemid the rate of proliferation of the cells fell. The number of cells taken from the Carrel's flask on the 4th-6th day after seeding was from two to four times smaller for the L-53 strain grown in the presence of colcemid (70th-89th subculture) than for the original strain. The slowing of proliferation of resistant cells subcultured in the presence of the selective agent has also been described for other resistant strains [3, 8]. The causes of this phenomenon are not completely clear and further investigations will be necessary to explain them.

To obtain some idea of the mechanisms responsible for the resistance of the L-53 cells to colcemid their sensitivity to other metaphase inhibitors was tested. Table 1 shows that substrain L-53 was highly resistant to Vinca alkaloids: to cause disappearance of nearly all the postmetaphase stages of mitosis in the L-53 population, the doses of these compounds had to be 100 times greater than for L cells. The level of resistance to colcemid and Vinca alkaloids was thus about equal. The L-53 cells also acquired some, although not much, resistance to the mitostatic action of 17β-estradiol (Table 1). The simultaneous appearance of resistance to different metaphase inhibitors sheds some light on the mechanisms of this resistance. This change in sensitivity is evidently explained not by a change in the protein of the microtubules, as was hitherto considered [1], but by other causes. The elucidation of these causes requires further investigations.

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