Exogenous MMTV is known to circulate by the lymphogenous route; expression of virus protein can be found in the thymus of C3H mice of a certain age [3], and it thus seems likely that antibodies against an unknown viral product may be present in the allogeneic serum. There are also molecular-genetic grounds for postulating transduction: the discovery of a sequence capable of coding a 26 kilodalton polypeptide in the terminal repetition of the virus and also the presence in the virion of a 14S RNA, with sequences common with the virus, but coding proteins having no common antigenic determinants with the virion [8].

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EXPRESSION OF THE TRANSFORMED PHENOTYPE IN COLCHICINE-RESISTANT TUMOR CELLS

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The writer showed previously that the development of resistance to actinomycin D (AD) in Djungarian hamster tumor cells cultured in vitro leads to changes in the expression of malignant transformation in these cells [2]. This takes the form of a decrease in the ability of the cells to survive transplantation and to grow without anchorage to the substrate (a decrease in the frequency of colonies in semisolid medium).

Resistance to AD is connected with a change in permeability of the plasma membrane for the antibiotic and with the appearance of homogeneously stained regions (HSR) in the chromosomes [8]. These HSR, as was shown previously for cells resistant to colchicine and methotrexate [5, 11], are the cytological expression of gene amplification.

These observations suggested that the development of resistance to other chemical substances also, resistance to which is characterized by disturbance of plasma membrane permeability and by gene amplification, would also lead to changes in expression of tumor cell transformation.

In the present investigation expression of transformation was studied in cultures of tumor cells resistant to the mitostatic poison colchicine, resistance to which is determined by amplification of genes coding, prob-

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TABLE 1. Transplantability and Growth in Semisolid Medium of Colchicine-Resistant Cells

Culture	Selective dose of colchicine	TD_{50}	Mean latent period of appearance of tumors, days (dose 10^6)	Cloning efficiency in agar, 10^{-1}
DM-15	0	$4{,}0 \cdot 10^3$	10,2±0,4	3,1±0,4
$DM^{0,1/1}$	0,1	$4{,}0 \cdot 10^3$	12,8±2,4	—
$DM^{5/1}$	5,0	$3{,}0 \cdot 10^5$	41,9±4,3	2,2±0,4
$DM^{0.1/2}$	0,1	$1{,}0 \cdot 10^4$	16,9±1,1	2,4±0,4
$DM^{5/2}$	5,0	$3{,}0 \cdot 10^4$	18,3±1,1	1,2±0,7
DM^{cap}	0	$3{,}8 \cdot 10^3$	16,4±2,9	6,6±0,8
$DM^{cap0,1/3}$	0,1	$8{,}0 \cdot 10^4$	14,5±1,7	1,4±0,2
$DM^{capl/4}$	1,0	$3{,}0 \cdot 10^5$	24,2±2,8	3,0±0,7
$DM^{cap0,1/5}$	0,1	$1{,}0 \cdot 10^4$	24,1±8,6	2,2±0,3
$DM^{cap0,1/5\ w/o\ c}$	0	$1{,}0 \cdot 10^3$	12,9±1,2	4,0±0,5

Legend. w/o c – without colchicine.

ably, low-molecular-weight protein p20 [5]. The ability of these cells to grow in the form of tumors when inoculated into animals and to form colonies in semisolid medium without anchorage to a solid substrate was studied.

EXPERIMENTAL METHOD

Experiments were carried out on Djungarian hamster cell lines resistant to 0.1-5.0 μg/ml colchicine, which were obtained by B. P. Kopnin [4, 5]. These cultures are derivatives of line DM-15 and are embryonic fibroblasts transformed by virus SV40, carrying the genetic defect of absence of hypoxanthine-phosphoribosyltransferase activity [3]. A series of cell cultures, designated by the superscript "cap" carries resistance to chloramphenicol, evidently due to a mutation in mitochondrial DNA [6].

The cells were grown on Eagle's medium with the addition of 10% bovine serum and selective agents: 0.1-5.0 μg/ml colchicine and 15 μg/ml 6-mercaptopurine.

Cells of the initial and colchicine-resistant cultures were inoculated subcutaneously into newborn Djungarian hamsters of our own rearing, in doses of 10^2-10^6 cells. Ten to 15 animals were inoculated with each dose. The animals were examined daily to determine the latent period of appearance of tumors. On the basis of data on the number of tumors appearing after injection of the different doses of cells, TD_{50} was determined (the dose of cells with which tumors appeared in 50% of animals).

The ability of the cells to grow independently of the substrate was determined by growing the cells in medium with semisolid agar, as described previously [1]. The number of cells seeded per petri dish 35 mm in diameter was 10^2-10^3. The colonies were counted after culture of the cells for 12-14 days at 37°C in an atmosphere with 5% CO_2.

EXPERIMENTAL RESULTS

Cells of lines DM-15 and DM^{cap} were highly transformed cultures growing actively in vitro. These cells formed tumors on inoculation in small doses, i.e., they are highly malignant (Table 1). Both lines formed colonies with high frequency in medium with semisolid agar, evidence of a high degree of expression of substrate independence of growth in these cells.

Cultures isolated after culture of the cells in medium with low doses of colchicine (0.1 μg/ml), namely $DM^{0\cdot1/1}$, $CM^{0\cdot1/2}$, and $DM^{cap\ 0\cdot1/3}$, were almost or completely indistinguishable in rate of successful inoculation from the original lines. The cloning efficiency of cells of these lines in medium with semisolid agar was high: The frequency of colonies was $(1.4\text{-}2.4) \times 10^{-1}$.

An increase in the degree of resistance of the cells to colchicine (selective doses up to 1-5 μg/ml) led to a more (lines $DM^{5/1}$, $DM^{cap\ 1/4}$) or less (line $DM^{5/2}$) considerable decrease in the rate of successful inoculation.

The frequency of colonies in medium with semisolid agar remained high with these resistant cultures (Table 1).

Analysis of transplantability of the cells and their ability to grow independently of a substrate thus showed that resistance to low doses of colchicine did not affect, or affected only slightly, expression of the basic feature

of the transformed cell phenotype, namely ability to grow in the form of tumors when inoculated into animals. Resistance to high doses of colchicine was accompanied by a decrease in transplantability. Substrate-independent growth was expressed equally in cultures with low and with high levels of resistance to colchicine.

The level of resistance in cells isolated in low concentrations of colchicine has been shown [5] to be increased by 15-20 times, whereas in cultures obtained as a result of exposure to high selective doses of the agent, resistance to colchicine was increased by 200-800 times. Our own data, obtained by analysis of colchicine-resistant cultures, show that a change in transplantability is observed in cells with a high level of resistance and that this effect is not observed when the level of resistance is low.

Line $DM^{5/2}$ differed somewhat in transplantability. The high degree of successful inoculation of cells of this line could be attributable to the fact that this culture is polyploid, whereas the other two, with the same level of resistance, are near-diploid. This hypothesis is based on data in the literature indicating that the transplantability of polyploid cells in culture may be significantly higher than that of near-diploid cells [10].

It has been shown for colchicine-resistant cultures that resistance to this substance is an unstable characteristic [7]. Culture in medium without the selective agent leads to loss of resistance. Our own data on transplantation of cells which have lost their resistance to colchicine show that loss of resistance leads to an increase in transplantability up to a level characteristic of the original cells (Table 1, line $DM^{cap\ 0{\cdot}1/5}$ w/o c). Similar restoration of transplantability was observed in Djungarian hamster cells which had lost their resistance to AD [2].

With the appearance in tumor cells of resistance to chemicals, resistance to which is connected with a disturbance of permeability of the plasma membrane for these substances and with gene amplification, a number of characteristics of the tumor cells may be changed. This is expressed primarily as a decrease in transplantability of the cells into animals.

Resistance of this type does not always affect expression of substrate independence of growth: Whereas expression of this characteristic in cells resistant to AD is reduced, during development of resistance to colchicine the frequency of colonies in semisolid medium is not reduced.

The study of the effect of gene amplification in the development of resistance of tumor cells to chemicals on expression of transformation is interesting in connection with the study of mechanisms of development of resistance to antitumor preparations and it calls for further investigation.

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