TRANSFORMATION OF TISSUES OF THE HAMSTER BY THE ACTION OF MONKEY VIRUS 40 AND AS A RESULT OF PROLONGED CULTIVATION IN VITRO

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UDC 576.8.095.383:576.858.093.
35+616-006-0929-02:576.858

Several writers have described the transformation of tissue cells of hamster embryos [4, 6] and of human diploid cells [5] under the influence of monkey virus 40 (MV_{40}). The transformation of human diploid cells was found to be directly dependent on the dose of virus. Implantation of the transformed hamster tissue cultures into adult hamsters [4, 6] led to the development of malignant tumors.

In this investigation the effect of the concentration of MV_{40} on transformation of hamster embryonic tissue was studied and the characteristics of "spontaneous" transformation and of transformation by the action of monkey virus were compared.

EXPERIMENTAL

Strain MV_{40} No. A-426 (National Institutes of Health, U.S.A.) was used in the experiments. To obtain the transforming effect, a suspension of hamster embryonic cells (2nd-6th passages) was infected with MV_{40}. After contact for 30 min at 37° between virus and cells the mixture was transferred to flasks. Unadsorbed virus was washed off the cells after 18-20 h. The cultures were transplanted every 3-4 days. The cells were grown on Eagle's medium with 10% bovine serum. MV_{40} was isolated from the cultures in primary kidney cultures of green guenon cells. The titer of the virus was determined by the plaque method [2].

The virus antigen was localized by Coons' direct fluorescent antibody method, using a hyperimmune rabbit MV_{40} antiserum conjugated with fluorescein isothiocyanate. The method of impression preparations was used for the karyologic investigations [3] and the chromosomes were counted in not less than 50 cells of each culture. The preparations for the cytological investigation were obtained in the usual way. The oncogenic activity of the cells was tested in 3-6 week old Siberian hamsters. The cells were injected into the subepithelial layer of the retrobuccal pouches and subcutaneously in the region of the spine.

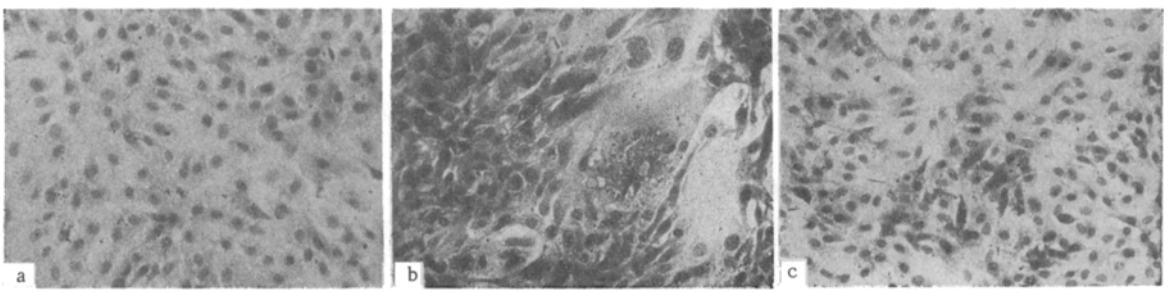

Fig. 1. General appearance of control and transformed cultures of hamster embryonic cells. a) Control culture of hamster embryonic cells (17th passage); b) culture transformed by MV_{40}, 26th passage after infection; c) "spontaneously" transformed culture (41st passage). Hematoxylin-eosin. 180×.

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(Presented by Active Member of the Academy of Medical Sciences of the USSR M. P. Chumakov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 6, pp. 118-122, June, 1967. Original article submitted November 30, 1965.

TABLE 1. Characteristics of Cultures of Hamster Embryonic Cells Infected with Different Doses of MV_{40} Indicated by Number of Nucleoli and of Giant Cells (in %)

No. of nucleoli and presence of giant cells	Time after infection								
	1st passage (6th day)				6th passage (25th day)				
	dilution of virus								
	undiluted	10^{-1}	10^{-2}—10^{-5}	cont. without virus	undiluted	10^{-1}	10^{-2}	10^{-3}—10^{-5}	cont. without virus
1-3 Nucleoli	50	61,8	66,8—68,6	70,3	28,6	59,8	60,8	65,2—72,4	69,8
3-5 Nucleoli	43,6	32,8	28,8—29,6	27,7	20,3	33,0	32,7	26,0—29,6	26,1
More than 5 nucleoli	6,4	5,4	2,5—3,8	2,0	36,1	7,2	6,5	2,4—5,1	5,1
Giant cells with fragmented nucleoli	0	0	0	0	15	0	0	0	0

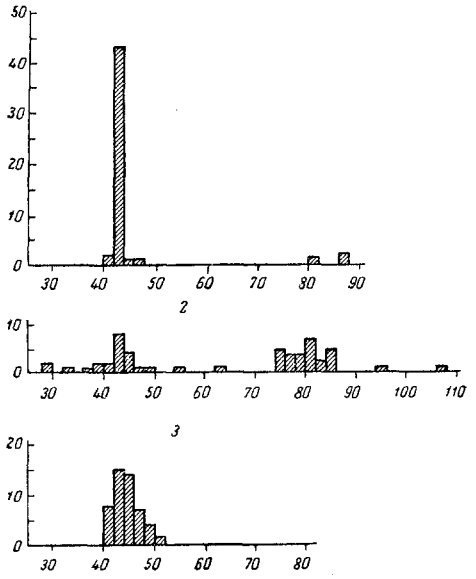

Fig. 2. Histograms of control and transformed cultures of embryonic hamster tissue. 1) Control culture; 2) culture transformed by MV_{40}; 3) "spontaneously" transformed culture. Ordinate) number of cells; abscissa) number of chromosomes in the cell.

EXPERIMENTAL RESULTS

The cell strains of the hamster embryonic cultures in the experimental conditions used could be maintained as a rule not more than 2 months. The cultures consisted of long and polygonal cells, giving a continuous monolayer by the 3rd-4th day after passage. However, after 1.5-2 months, growth of the cells became much slower and the strain died. Transformation could be carried out throughout this period in the tissue cultures infected with MV_{40}.

To determine the minimal transforming dose three experiments were carried out in which the maximal concentration of virus varied from 4 PFU (plaque-forming units) per 10 cells to 10 PFU per cell. The subsequent infecting doses were tenfold dilutions to 10^{-6}.

The first signs of transformation (the faster acidification of the medium, the appearance of giant cells with deformed nuclei in the culture) were observed 20-25 days after infection.

Small islands of intensified growth of cells appeared after 30-35 days in the monolayer, and they rapidly proliferated, while the cells of the monolayer gradually died. After 6 days of growth, a tendency for the number of nucleoli to increase was observed in the cultures infected with maximal doses of virus, and this was especially marked at the 6th passage (Table 1). The number of giant cells by this time had reached 15-20% (1000 cells were counted).

The cultures transformed under the influence of the virus were characterized by considerable polymorphism, a high content of giant cells with large, deformed nuclei and multiple nucleoli, and by high mitotic activity with numerous polycentric and anomalous mitoses. No intranuclear inclusions could be seen, and cytoplasmic inclusions were observed equally frequently in the control culture and the culture infected with virus.

The structure of the control and the transformed culture may be seen in Fig. 1a and b.

The minimal doses of virus causing transformation for 2 months were 1 PFU per 1, 10, and 25 cells (for the 3 corresponding experiments). The tissue cultures infected with smaller doses of virus possessed characteristics identical with those of the uninfected control cultures during 2 months of observation.

TABLE 2. Results of Implantation of Tissue Culture Cells into 3-6 Week Old Hamsters

Tissue cultures	Passage	Mode of injection	Dose of cells	No. of tumors*	Time after appearance of tumors (in days)	Time of observation (in days)
Control culture of hamster embryonic cells	4	Into retrobuccal pouch	3.4×10^6	0/2	–	180
The same	16	Subcutaneously in dorsal region	10^3-10^6	0/12	–	120
		Into retrobuccal pouch	10^3-10^6	0/8	–	120
"Spontaneously" transformed culture	42	Subcutaneously in dorsal region	10^3-10^6	0/12	–	120
		Into retrobuccal pouch	10^3-10^6	0/7	–	120
Experiment 1 Culture transformed by MV_{40}	19	The same	3×10^3	2/2	60–25	45
Experiment 2	28	" "	10^6	2/2	9	60
			10^4	2/2	15	68
			10^3	2/2	30	90
			10^2	2/2	100–110	152
			10	0/2	–	165
		Subcutaneously	10^4	3/3	25	70
			10^3	3/3	42	70
			10^2	3/3	50	70
			10	1/3	60	80
Experiment 3 †	20	Into retrobuccal pouch	1.3×10^4	2/2	18	36
			1.3×10^3	2/2	26	36
			1.3×10^2	2/2	30	50
			1.3×10	2/2	35	50

*Numerator–number of animals with tumors; denominator–number of infected animals.
†The animals received three intramuscular injections, each of 2.5 ml cortisone; the first at implantation of the cells, the second and third at intervals of 3 days.

Chromosome counts revealed considerable changes in their number in the cells of the transformed cultures: only 16% of the cells had the specific number of chromosomes and the rest were aneuploid. The number of tetraploids and near-tetraploids was appreciably increased.

Histograms of the control and transformed cultures are given in Fig. 2.

Besides the tissue lines becoming capable of prolonged cultivation after transformation under the influence of MV_{40}, one strain of uninfected hamster embryonic tissue was obtained which has so far completed 65 passages (growth for 6 months). This "spontaneously" transformed strain consists of fibroblast-like cells (Fig. 1c), 72.8% of which have from 1 to 3 nucleoli, but only 1.8% have more than 5. No giant cells are present. The morphological characteristics of the control culture were similar.

However, chromosome counts showed a change in their number: 70% of the cells were aneuploid, but no tetraploid or near-tetraploid cells were present.

All the transformed cell strains, and also the control cultures, were implanted into 3-6 week old hamsters. The results of these experiments showed a marked difference in the oncogenic properties of the cells of the cultures transformed by the action of the virus and of the "spontaneously" transformed cultures (Table 2).

The cultures not infected with MV_{40} did not produce tumors even after implantation of 1 million cells, whereas only from 10 to 100 cells of the virus-transformed culture were required to produce a tumor in the animals after 35-100 days. The latent period correlated with the number of implanted cells. Histological investigation of three tumors, carried out by A. P. Savinov, showed that they were fibrosarcomas. The tumor cells were easily transplanted both in vivo and in vitro. In the latter case, the tissue cultures were indistinguishable morphologically from the original transformed culture.

Investigation of the tissue cultures for their virus content, whether by the plaque method or by testing their cytopathogenic effect on kidney cells of the green guenon, demonstrated the presence of virus only in the cells of a hamster embryo infected with the maximal dose of MV_{40} (1 PFU per cell). Virus was found only on the 3rd day after infection, and thereafter, none could be isolated. In the control cultures infected with small doses of MV_{40}, no virus was found by these methods. Specific fluorescence of the virus antigen after staining with labeled serum occurred in the first passage only in the tissue culture from which MV_{40} was isolated.

No virus was found in the "spontaneously" transformed culture. The results show that hamster embryonic tissue can be transformed by the action of a high dose of MV_{40} of similar magnitude to the dose for human diploid cells.

The cells transformed by the action of the virus differed sharply in their oncogenic properties from the cells of the "spontaneously" transformed strain. Transformation of the cells under the influence of prolonged cultivation in vitro [1] is not always accompanied by malignant change, for it would be difficult to explain the negative results in the experiments described above by immunogenetic incompatibility between the injected cells and the recipient or by inoculation of too few cells.

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