CHROMOSOMAL ABERRATIONS INDUCED BY SV 40 T ANTIGEN INTRODUCED INTO CELLS BY MEANS OF LIPOSOMES

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UDC 616-018.1:572.224.23]-02:
578.827.22]-07

KEY WORDS: SV 40 virus; T antigen; chromosomal aberrations; liposomes.

It is nowadays considered that activation of cellular pathogenic oncogenes is a general pathogenetic mechanism of neoplastic transformation. It arises not only in gene mutations [15], but also in structural changes induced in cell DNA [1, 12, 14] by various chemical, physical, or biological (viral) agents. In our view induced somatic recombination is a general pathogenetic mechanism which combines carcinogenic factors of varied etiology [8].

Lesions of the chromosomal apparatus of cells are found when they are infected by oncogenic viruses. In particular, it has been shown that virus SV 40 can induce chromosomal aberrations and gene mutations in Chinese hamster cells in the presence of high multiplicity of infection [6]; this effect, moreover, has been linked with activity of a virus protein, namely T antigen [2]. The writers showed previously that chromosomal aberrations arise in cells transformed by tsA mutants of SV 40 virus throughout the period of cell culture only at a temperature of 33°C, which is permissive for T antigen [10].

In the investigation described below the ability of highly purified T antigen to induce chromosomal aberrations was studied after introduction into cells with the aid of liposomes.

EXPERIMENTAL METHOD

T antigen of SV 40 virus was purified by the method developed previously [3, 11]. Liposomes containing T antigen were obtained by the phase reversal method. Their diameter was 0.2-0.4 μ and they were resistant to the lytic action of serum [5]. To study chromosomal aberrations, Dzungarian hamster (Phodopus sungorus Pall.) cells, obtained from O. I. Sokova (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR), were used. The cells were grown on Carrell's flasks, washed off with Hanks' solution, and added to 1 ml of a suspension of liposomes (220 μmoles of phospholipid per flask) and incubated at 37° for 70 min, with periodic shaking of the vessel. The residue of liposomes was drawn off and the cells washed with medium without serum, and treated with the necessary quantity of nutrient medium with 10% serum. Cytogenic analysis was undertaken in the usual way. No fewer than 50 metaphase plates were analyzed at each point, in two parallel experiments.

EXPERIMENTAL RESULTS

The process of penetration of the T antigen and its distribution in the cells were studied by immunofluorescence. After 3 h T antigen was detected in the cytoplasm in the form of diffuse, or sometimes granular, fluorescence, which was concentrated after 5 h in the perinuclear zone, and revealed 10 h after the beginning of the experiment in the nuclei of 60-80% of the cells, in which it remained for about 24 h (Fig. 1).

The results of the action of purified T antigen on the state of the chromosomal apparatus are shown in Table 1. In the control culture, not treated in any way except to change the medium, 12% of metaphase plates with single chromosomal aberrations were found 24 h after the beginning of the experiment. The same picture was observed in cells treated with liposomes not containing T antigen, but containing all the other components, including serum albumin (BSA) as protein stabilizer. When the cells were treated with T antigen the percentage of aberrant metaphases rose to 50, and in some experiments higher still, to 70. Its mean value in four

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Laboratory of Biochemistry of Tumors, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 5, pp. 600-602, May, 1985. Original article submitted February 13, 1984.

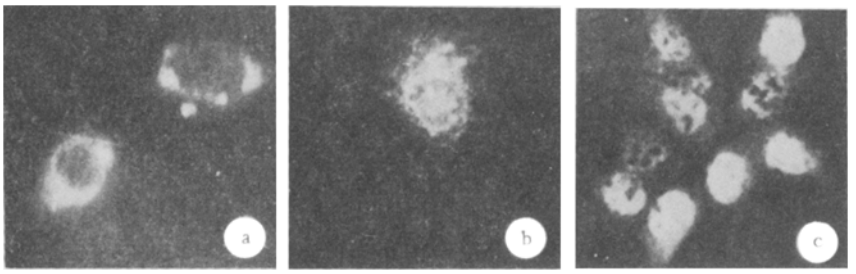

Fig. 1. Immunofluorescence detection of T antigen of SV 40 virus introduced into cells by means of liposomes: a) 3 h, b) 5 h, c) 10 h after introduction.

TABLE 1. Chromosomal Aberrations in 0-1552 Cells under the Influence of Purified T Antigen Introduced by Means of Liposomes

Parameter	Duration of exposure, days								
	1			2			5		
	control	liposomes		control	liposomes		control	liposomes	
		with BSA	with T antigen		with BSA	with T antigen		with BSA	with T antigen
Number of metaphases analyzed	100	150	200	150	100	150	100	100	100
Percentage of aberrant metaphase	12±2,8	14±1,6	58±9,9	8±2	13±4,2	37±3	9,5±2,1	12±1,4	12±2,8
Number of injuries per cell	0,12	0,15	0,76	0,08	01,5	0,52	0,09	0,12	0,15

TABLE 2. Changes in Ability of T Antigen to Induce Chromosomal Aberrations on Removal from the Preparation by Specific Immunosorbent

Parameter	Original T antigen	T antigen treated with Sepharose without antibodies, $1/2$ of volume	T antigen treated with immunosorbent		Control	
			$1/4$ of volume	$1/2$ of volume	liposomes without T antigen	original culture
Concentration of T antigen in preparation, CFT units/ml	560	280	20	<10	—	—
Percentage of aberrant metaphases	72±8,4	56±7,1	36±5,6	22±2,8	14±1,6	12±2,8

independent experiments was 58±9.9. Multiple chromosomal injuries were observed. On the 2nd day the number of chromosomal aberrations and injuries per cell was reduced somewhat, and by the 5th day this parameter was identical in value with the control. The action of T antigen was thus short in duration. As was observed above, the T antigen disappeared from the nuclei by the end of the 1st day, and after this its effect diminished, possibly as a result of its single hit action on the genetic apparatus of the cell.

To confirm that the observed effect depended on T antigen, before incorporating the material in the liposome we removed this protein from it with the aid of a specific immunosorbent (Sepharose with antibodies against T antigen bound to it [9]). The change in content of T antigen in the preparations was determined by the complement fixation test (CFT, Table 2). Addition of increasing doses of the immunosorbent led to partial or virtually complete removal of T antigen from the material subsequently incorporated into the liposomes. The results of treatment with the preparation showed corresponding changes: after removal of T antigen the percentage of aberrant metaphases fell from 72 to 36 and 22.

The commonest chromosomal injuries observed were aberrations of chromatid type: breaks, deletions, translocations, and gaps. Aberrations of chromosomal type were found less frequently: isobreaks, dicentrics, gaps. Under the influence of T antigen aberrations connected with structural changes in the chromosomes (translocations and dicentric chromosomes) were observed much more often than in the control cultures.

In cells exposed to the action of T antigen no chromosomal aberrations specific for it could be detected, evidence of the random character of the injuries induced by it. This is in agreement with data obtained earlier,

showing that T antigen can interact with different sites of cell DNA and chromatin [4, 7]. However, during selective pressure on the host organism, the formation of a cell clone with advantages over the rest, and leading to tumor growth, is very probable. In that case specific translocation of the oncogene to the zone of active chromatin, as has been shown for the myc gene in Burkitt's lymphoma [12], may be the factor triggering carcinogenesis.

Experimental data confirming the multistage nature of carcinogenesis have now been obtained. It is based on the successive incorporation and cooperative action of several cell oncogenes [13]. Very probably during transformation by SV 40 virus it is the T antigen that will be the factor in evolution of the tumor cell that is responsible for subsequent incorporation of cellular oncogenes with the aid of structural changes in cell DNA induced by it.

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