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Virogenic Hamster Tumor Cells: Induction of Virus Synthesis

Abstract. *Virogenic cell cultures of a hamster ependymoma originally produced by Simian Virus 40 were treated with proflavine, hydrogen peroxide, or mitomycin C. Induction of virus synthesis was observed. Similar experiments with hamster cells transformed by adenovirus type 12 or polyoma virus gave negative results.*

The properties of cells of hamster ependymomas produced by intracerebral inoculation of newborn animals with Simian Virus 40 (SV_{40}) have been described (*1*). No infectious virus was detectable in cell-free extracts of both primary and tissue-cultured tumor cells. However, when intact, viable tumor cells were seeded onto sensitive indicator cells (cultures from kidneys of African green monkeys), characteristic viral lesions appeared in the indicator cells adjacent to the tumor cells. It was postulated that the tumor cells harbored a persistent subviral infection which is transmissible to indicator cells by cell-to-cell transfer of subviral units. The term "virogenic state" was suggested to describe this virus-cell relationship.

When our attempts to induce virus synthesis in virogenic cells by exposure to ionizing radiation gave irregular results, we explored other means of virus induction. The results of such studies with proflavine, hydrogen peroxide, and mitomycin C are the subject of this report.

Three transformed hamster cell cultures free of detectable virus were employed in this study. They were the EP_A cell line derived from an ependymoma produced in hamsters by SV_{40}, a tissue culture preparation of a primary hamster sarcoma produced by adenovirus type 12 (*2*), and the TC-1 line of Stoker, a hamster kidney cell line transformed in vitro by polyoma virus (*2*).

Cell-free preparations of these malignant cells showed no evidence of infectious virus when tested in cultures of appropriate indicator cells. Tests for subviral infectivity were performed by seeding intact malignant cells directly on indicator cell cultures. After 8 and 14 days of incubation, the mixed cultures were disrupted by freezing and thawing and ultrasonic treatment. The cell-free preparations were assayed for presence of virus. As seen in Table 1, only cells transformed by SV_{40} gave evidence of persistent subviral infectivity.

In attempts to induce viral synthesis in these transformed cells we evaluated the effects of proflavine, hydrogen peroxide, and mitomycin C. Monolayer tube cultures of transformed cells were treated as shown in Table 2. The medium used in all experiments consisted of Eagle's minimum essential medium (*3*) containing 2 percent calf serum. Proflavine treatment was carried out in subdued light, and the treated cultures were shielded from light during the entire period of incubation. At 6 to 24 hours after treatment, slight cytotoxic effects were noticed in cultures treated with proflavine or mitomycin C. These cultures were thoroughly washed and incubated with drug-free maintenance medium. The cultures treated with hydrogen peroxide showed no significant toxic changes and were not refed. All cultures were placed on roller drums and were maintained with 2 ml of medium per tube at 36°C. Eight to ten days after treatment, the cells were frozen, thawed, and treated with high-frequency sound. The cell-free fluids were tested for the presence of virus. The results (Table 2) show the successful induction of virus synthesis in tumors produced by SV_{40}. The relatively small amount of virus recovered from induced cultures suggests that only a fraction of the virogenic cells were inducible under the present experimental conditions. By contrast, none of the treatments resulted in virus induction in the cells transformed by adeno- or polyoma virus. Failure to induce virus production in cells transformed by polyoma virus confirms previously published findings (*4*). Other investigators (*5*) have shown that "virus-free" tumors produced by SV_{40}, polyoma, or adenovirus type 12 release complement-fixing antigens specific for the virus or tumor.

Table 1. Detection of subviral infectivity in transformed hamster cells.

Hamster cells transformed by	Cell extracts	Intact cells seeded onto	Infectivity
SV_{40}	Negative	GMK*	Positive
Adenovirus type 12	Negative	HEK*	Negative
Polyoma virus	Negative	ME*	Negative

* GMK, green monkey kidney; HEK, human embryonic kidney; ME, mouse embryo.

Table 2. Attempts to induce virus production in transformed hamster cells.

Dose	Period of treatment	$TCID_{50}$* per culture of cells transformed by:		
		SV_{40}	Adeno-12	Polyoma
	No treatment			
None		0	0	0
	Proflavine			
1–5 μg/ml	6–8 hr	2	0	0
	H_2O_2			
$9 \times 10^{-5} M$	6–8 days	15	0	0
	Mitomycin C			
0.2–0.4 μg/ml	7–24 hr	10	0	0

* Fifty percent tissue culture infective dose.

The observations recorded in this study together with the evidence on complement-fixing antigens suggest a basic difference among cells transformed by these three oncogenic viruses with respect to the nature of the viral genetic material and its relationship to cellular DNA.

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