Transformation of Rodent Cells by Simian Adenovirus SA-7* (34127)

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Simian adenovirus SA-7 is highly oncogenic in hamsters (1) and transforms mouse, hamster, and rat cells *in vitro* (2–4). When assayed in primary hamster embryo cell cultures, SA-7 transforms the cells more efficiently [has a lower plaque-forming unit (PFU) to focus-forming unit (FFU) ratio] than human adenovirus type 12 (4). This report describes the SA-7 transformation of NIL-2 cells, a cloned line of hamster embryo cells, and of primary rat embryo cells and compares the efficiency of transformation of these cells with that of other adenoviruses.

Methods. Viruses. SA-7 virus, C8 strain, was obtained from Dr. H. Malherbe, Johannesburg, South Africa. It was passaged in the BS-C-1 line of African green monkey kidney cells (5) and plaque assayed on primary African green monkey kidney cells. Virus particle counts were made by Dr. E. A. Follett using an electron microscope (6). No adenovirus-associated virus was detected in the virus stocks by electron microscopy.

Cells. The hamster cell line NIL-2 was obtained from Dr. L. Diamond, Wistar Institute, Philadelphia, Pennsylvania. A clone derived from a single cell isolated by micromanipulation was used for these studies.

Rat embryo cells were isolated by trypsin dispersion of near-term Hooded Wistar or Fischer rat embryos and used in primary tissue culture passage.

Transformation assays and other methods. The assay methods in monolayer cultures and in agar suspension cultures of NIL-2 cells and rat embryo cells have been described (7) as have the methods for carrying out histological studies of tumors and colonies of transformed cells (8), serological tests (3), tests for infectious virus in transformed cells (9), and transplantation assays (3).

Results. Transformation of NIL-2 and rat embryo cells. When monolayers of NIL-2 or rat embryo cells were exposed to SA-7 virus, foci of multilayered cell growth were visible 10 and 23 days after infection, respectively. The foci gradually increased in size and were easily recognized against a monolayer background of untransformed cells (Figs. 1, 2).

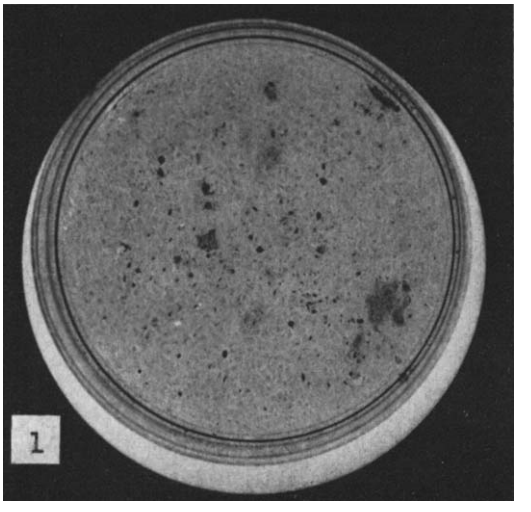

FIG. 1. Culture of NIL-2 cells showing foci of transformed cells 25 days after infection; Giemsa stain; actual size.

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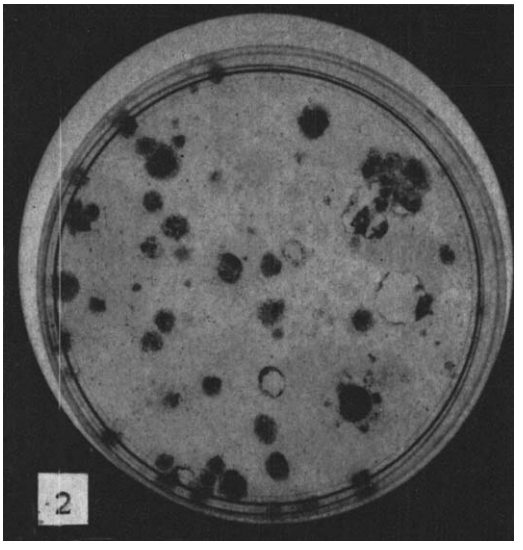

FIG. 2. Culture of rat embryo cells showing foci of transformed cells 35 days after infection; Giemsa stain; actual size.

The foci were similar in appearance to those induced in these cells by adenovirus types 1, 2, 3, and 12 (7, 9–11). No areas of morphologically altered cells were seen in uninoculated control cultures. The results of representative experiments are summarized in Table I.

When a suspension of NIL-2 cells was exposed to virus (approximately 2.5 PFU/cell) and 7.5×10^5 cells were plated in agar suspension cultures, colonies 0.1–0.2 mm in diameter formed after 21 days' incubation (average 64 colonies/dish in two experiments) and resembled those of adenovirus-12 tumor cells (8) or transformed cells (7). No colonies formed in the control cultures seeded with uninfected NIL-2 cells.

TABLE I. Transformation of NIL-2 Cells and Rat Embryo Cells by SA-7.[a]

NIL-2 cells[b]		Rat embryo cells[c]	
Virus input PFU/cell	Foci/dish	Virus input PFU/cell	Foci/dish
Control	0, 0	Control	0, 0, 0, 0
166	TNC, TNC[d]	62.5	17, 10, 8, 5, 3, 3
33	136, 173, 108	12.5	5, 1, 2, 2, 0, 0
8.3	25, 34, 63	3.1	2, 1, 1, 0, 0, 0

[a] SA-7 C8 strain: 1.8×10^9 total virus particles /ml and 2.5×10^8 PFU/ml.

[b] 1.5×10^6 cells per culture exposed to virus.

[c] 4×10^6 cells per culture exposed to virus.

[d] TNC = too numerous to count.

Cell line derivation. Colonies of transformed NIL-2 cells were picked from agar with finely drawn Pasteur pipettes and subcultivated in plastic Petri dishes containing Eagle's medium supplemented with 10% fetal calf serum. The colonies attached to the surface of the dishes and yielded cell lines.

Foci of transformed cells from monolayer cultures of rat cells were also picked with Pasteur pipettes and cultured in dishes in the same medium as the colonies and yielded cell lines with an epithelioid morphology typical of adenovirus-transformed cells.

Cultivation in agar medium. One line of SA-7–transformed NIL-2 cells in seventh passage formed colonies in agar with a plating efficiency of 9.4%. The histology of the colonies was typical of adenovirus-induced hamster tumors (Fig. 3) (2, 8).

Antigenic analysis of SA-7–transformed NIL-2 cells. Results of complement-fixation reactions are summarized in Table II. SA-7 tumor antigen was present in the transformed cells; other tumor antigens (SV-20, SV_{40}, Ad-7, Ad-12) were not present.

Transplantation studies. When newborn or

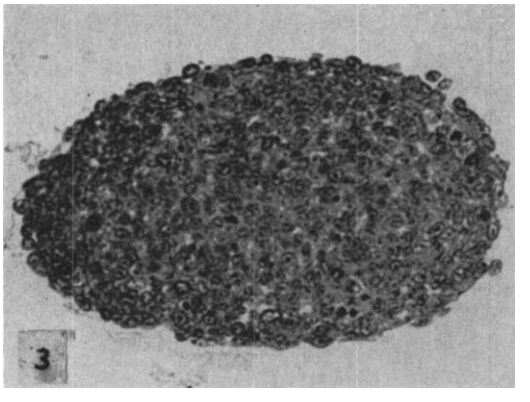

FIG. 3. Colony of SA-7–transformed NIL-2 cells from agar suspension culture. Compact and uniform cells somewhat less differentiated than those in the hamster tumor but consistent with anaplastic type of adenovirus-transformed cell; Hematoxylin and eosin stain; ×200.

TABLE II. Specificity of Adenovirus Tumor Antigen as Determined by Complement Fixation.

Antigen	Hamster tumor antisera					
	SA-7	SV-20	SV-40	Ad-7	Ad-12	Normal
SA-7–transformed NIL-2 cells	16[a]	0	0	0	0	0
SV-40–transformed hamster cells	0	0	1024	0	0	0
Ad-12–transformed hamster cells	ND[b]	ND	0	ND	256	0

[a] Reciprocal of complement-fixing antigen titer (20% cell extracts).
[b] ND = not done.

adolescent hamsters were injected with various numbers of SA-7–transformed NIL-2 cells, tumors developed at the site of injection within 2 months (Table III). Untransformed NIL-2 cells in twenty-second passage also formed tumors (10^6 cells = 8/8; 10^5 = 3/4; 10^4 = 0/2). In another experiment one of five adolescent hamsters developed a tumor after the inoculation of 10^5 untransformed NIL-2 cells.

Histological studies of hamster tumors. Tumors which formed in hamsters at the site of injection of SA-7–transformed NIL-2 cells were undifferentiated sarcomas resembling those induced in hamsters by SA-7 (2) or by human adenoviruses (8) (Fig. 4). The histologic characteristics of hamster tumors also resembled those of the cell colonies of SA-7–transformed NIL-2 cells (Fig. 3).

Comparison of transforming efficiency of various adenoviruses and the hybrid virus adeno 7/SV_{40}. In these studies 3.1×10^4 infectious units of SA-7 were required per transformation in NIL-2 cells compared to 3.9×10^6 Ad-12 or 4.3×10^7 Ad-7 infectious units (7). Only Ad-7/SV_{40} (E46+) had a higher transformation efficiency, 2.7×10^4 infectious units per transformation (Table IV).

TABLE III. Occurrence of Tumors in Hamsters Inoculated with SA-7–Transformed Cells.[a]

No. of cells inoculated	Infants (2–3 days of age)	Adolescents (21 days of age)
10^6	5/5[b]	5/5
10^5	9/9	3/5
10^4	8/9	2/4
10^3	5/10	ND[c]

[a] Eleventh to fourteenth passage level.
[b] Number of animals with tumors/number of animals inoculated.
[c] ND = not done.

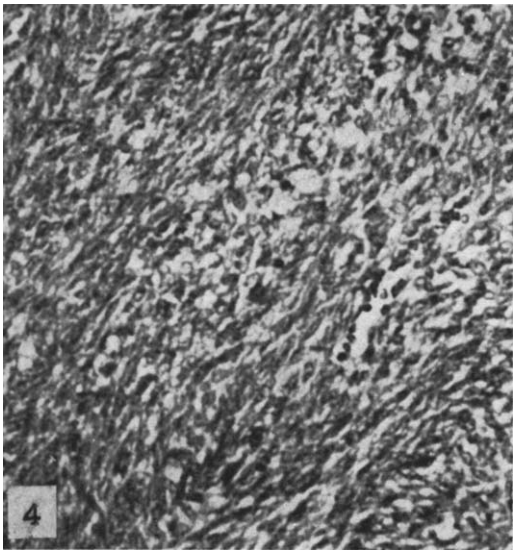

FIG. 4. Hamster tumor induced by SA-7–transformed NIL-2 cells is composed of closely packed uniform spindly cells with hyperchromatic nuclei. Morphologic picture, together with scattered tumor giant cells, resembles that seen in hamster tumors induced by SA-7 and human adenoviruses; Hematoxylin and eosin stain. ×200.

When assayed in rat embryo cells, SA-7, Ad-12, Ad-1, and Ad-7/SV_{40} had higher transforming efficiencies than Ad-2 or Ad-7.

Discussion. Casto has reported that 2.6×10^5 infectious units of SA-7 are required per transformation of primary hamster embryo cells (4). Our finding of 3.1×10^4 infectious virus units per transformation of NIL-2 cells is lower and perhaps related to differences between primary hamster cells and an established line of hamster cells. Previous studies

TABLE IV. Transformation of NIL-2 Cells and Rat Embryo Cells by Adenoviruses.[a]

Adenovirus		NIL-2 cells		Rat embryo cells	
		VP/FFU	PFU/FFU	VP/FFU	PFU/FFU
Group A	Ad-12	1.7×10^7	3.9×10^6	5×10^6	1.1×10^6
Group B	Ad-7	1.7×10^8	4.3×10^7	3.2×10^7	8.3×10^6
Group C	Ad-1	CPE	CPE	4.8×10^6	6.5×10^5
	Ad-2	CPE	CPE	2.4×10^8	4×10^7
SA-7		2.2×10^5	3.1×10^4	3.9×10^6	5.4×10^5
Ad-7/40		1.5×10^5	2.7×10^4	4×10^6	7.5×10^5

[a] CPE = cell sheet destroyed by viral cytopathic effect; VP = total virus particle count by electron microscopy; FFU = amount of virus to produce one transformed focus; PFU = amount of virus to produce one plaque.

have shown that *in vitro* transformation by polyoma and SV_{40} viruses is more efficient in established cell lines than in cells in primary or early tissue culture passage (12, 13). One line of our SA-7–transformed NIL-2 cells was shown by Magdalena Piña and Maurice Green to synthesize SA-7–specific RNA which formed RNAse-resistant complexes with SA-7 DNA. Therefore, both serological and biochemical evidence indicates that the NIL-2 cells were transformed by SA-7 virus.

Summary. A clone of the hamster cell line NIL-2 cells and primary rat embryo cells were transformed by simian adenovirus SA-7. The transformed NIL-2 cells formed foci of multilayered growth in monolayer cultures under agar and colonies when suspended in agar medium. The transformed rat cells formed foci under liquid medium. Cell lines were derived from both NIL-2 and rat transformed cells. A virus stock containing 1.8×10^9 particles and 2.5×10^8 PFU/ml contained 8×10^3 FFU/ml in NIL-2 cells and 4.6×10^2 FFU/ml in rat embryo cells. Approximately 3.1×10^4 infectious units were required to induce one focus in NIL-2 cells and 5.4×10^5 infectious units were required in rat embryo cells. The transformed NIL-2 cells contained SA-7–specific tumor antigens and RNA. They formed colonies in agar and induced tumors in hamsters with characteristic adenovirus-induced tumor pathology.

SA-7 transforms NIL-2 more efficiently than human adenovirus types 7 or 12; it transforms rat cells at approximately the same efficiency as types 1 and 12.

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