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A Fragment of the Simian Virus 40 Early Genome Can Induce Tumors in Nude Mice

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Cell lines transformed by simian virus 40 mutant F8*dl* (deleted from 0.168 to 0.424 map units, corresponding to the carboxy-terminal 62% of the wild-type simian virus 40 large tumor antigen) are tumorigenic in nude mice. Four of five C3H10T1/2 cell lines transformed by F8*dl* were tumorigenic in nude mice, whereas two of two wild-type transformants were tumorigenic.

The simian virus 40 (SV40) mutant F8*dl* lacks all of the DNA sequences between 0.168 and 0.424 map units, which in wild-type SV40 encode the carboxy-terminal 62% of the large tumor antigen (7). Although F8*dl* is defective for viral DNA replication (7), cloned DNA of this mutant can abortively transform Fisher rat F111 cells to anchorage-independent growth and can stably transform C3H10T1/2 mouse cells (6) in a focus assay (8, 9). The efficiency of stable transformation of these mouse cells is low (ca. 1% of that of wild-type SV40 DNA), but cell lines expanded from transformed foci contain integrated mutant DNA, express truncated forms of large T antigen, and exhibit a full range of transformed phenotypes, including the ability to grow while suspended in soft agar. Interestingly, in F8*dl*-transformed C3H10T1/2 cells, the stability of the 53,000-molecular-weight [53K] nonviral tumor antigen is similar to that found in the untransformed cell line (9). Thus, it appears that neither the large T viral DNA replication function nor the ability of large T antigen to stabilize the 53K antigen is required for SV40 to transform this cell line.

F8*dl*-transformed mouse lines appear identical to cells fully transformed by wild-type SV40 in their ability to grow in low serum concentrations, to high saturation densities, and in agar. Therefore, we might expect them to be tumorigenic. However, since F8*dl* has such an extensive deletion of the sequences that encode large T antigen and since F8*dl* transforms mouse cells with low efficiency, it seemed important to test the tumorigenicity of F8*dl* transformants.

We infected monolayers of C3H10T1/2 cells with cloned DNA of either wild-type SV40 or F8*dl* and expanded individual foci into cell lines (4, 10). Two wild-type and five mutant transformants were selected for study. In contrast to the parental untransformed line, all of these transformed cell lines grew in 1% fetal calf serum, reached high saturation densities, and grew in soft agar (Table 1). As expected, both wild-type-transformed lines expressed the SV40 large-T antigen, whereas only truncated forms of large T antigen could be detected in the mutant-transformed lines (9). All seven lines expressed the SV40 small t antigen.

For tumorigenicity studies, cells were harvested from culture by trypsinization, washed three times in phosphate-buffered saline, and injected subcutaneously into 6- to 8-week-old athymic BALB/c (nu/nu) mice (purchased from Harlan-Sprague-Dawley, Madison, Wis.). Tumor growth was monitored, visually and by palpation, weekly. Both of the wild-type SV40 DNA-transformed C98 and C95, and four of five F8*dl*-transformed cell lines, i.e., C49, C50, C51, and C53, formed progressive tumors. Except for C53, the F8*dl*-transformed cell lines appeared to grow into tumors with a latency period similar to that observed with wild-type SV40-transformed C3H10T1/2 lines. However, F8*dl*-transformed C32 and the nontransformed parental cell line C3H10T1/2 failed to form tumors in nude mice at a dosage of 10^8 cells per mouse (Table 2).

We prepared cell lines from tumors excised from the nude mice injected with mutant-transformed cells and used serum from an SV40-tumor-bearing hamster to immunoprecipitate [^{35}S]methionine-labeled proteins from cellular extracts. Figure 1 shows a fluorogram of these immunoprecipitates after

TABLE 1. Properties of cells transformed by F8*dl* or wild-type SV40

Clone	Transforming virus	Growth in low serum[a]	Saturation density (10^5 cells/cm^2)[b]	Growth in agar (%)[c]
10T1/2	Untransformed	–	0.5	0
95	Wild type	+	4.7	12
98	Wild type	+	3.6	8
32	F8*dl*	+	1.2	10
49	F8*dl*	+	2.5	15
50	F8*dl*	+	2.8	4
51	F8*dl*	+	3.4	36
53	F8*dl*	+	2.6	36

[a] Cells from each line were plated at 10^4 cells per 35-mm dish in Dulbecco modified Eagle medium plus 10% fetal calf serum. The next day, cells were washed and fed with Dulbecco modified Eagle medium plus 1% fetal calf serum. On days 4 and 7 after seeding, cells were trypsinized and counted in each culture. Untransformed 10T½ cells stopped growing by day 4 after seeding. Transformed cells were defined as those that continued to grow after day 4.

[b] Cells from each line were plated in 35-mm dishes at a density of 10^5 cells per dish in Dulbecco modified Eagle medium plus 10% fetal calf serum. Cells were counted on replicate plates every few days until saturation was reached or until the transformed cells began to detach from the monolayer. The detachment of transformed cells made exact quantitation of the saturation density difficult.

[c] Cells from each line were trypsinized, suspended in 0.32% agar–Dulbecco modified Eagle medium–10% calf serum, and plated in 60-mm dishes. After incubating the plates for about 2 weeks at 37°C, cultures were examined microscopically for the presence of transformed colonies. The percentage of cells that grew in agar was then calculated.

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TABLE 2. Tumorigenicity testing of F8*dl*-transformed and control cells in nude mice

Expt	Cell line	SV40 transformation	No. of cells injected	No. of mice with tumor/ no. of mice injected	Latency period
1	C3H10T1/2	None	6×10^7	0/2[a]	
	C98	WT[b]	9×10^7	2/2	2 wk
	C49	F8*dl*	5×10^7	2/2	3–4 wk
	C50	F8*dl*	3×10^7	2/2	3–4 wk
	C53	F8*dl*	1×10^8	2/2	2 wk
	C32	F8*dl*	1×10^8	0/2[a]	
2	C3H10T1/2	None	1×10^8	0/6[a]	
	C98	WT	3×10^7	2/2	3–4 wk
	C95	WT	6×10^7	1/1	3 wk
	C32	F8*dl*	1×10^8	0/5[a]	
	C51	F8*dl*	1×10^8	3/5[c]	2–4 mo

[a] These mice remained tumor-free for their lifetime, which was 4 to 8 months after injection.
[b] WT, Wild-type strain 776.
[c] Two tumor-free mice died 2 months after cell injection.

polyacrylamide gel electrophoresis. Small t antigen and the truncated forms of large T antigen present in the original transformed cell lines were detected in each of the tumor cell lines derived from nude mice, suggesting that one or more of these proteins is responsible for the tumorigenicity of F8*dl*. Constructs of SV40 deletion mutants encoding N-terminal fragments of large T antigen with (1, 2) or without small t antigen expression (2) have been shown by others to transform rat cells. To our knowledge, however, this is the first report that cells transformed by a small fragment of SV40 early region can be tumorigenic.

From these experiments, we conclude that F8*dl*, which lacks the SV40 sequences between 0.168 and 0.424 map units, can transform mouse cells to the full tumorigenic phenotype. We showed earlier that the levels of the 53K nonviral tumor antigen in F8*dl*-transformed C3H10T1/2 cells are similar to those found in the untransformed parental line (9). Since F8*dl*-transformed lines are tumorigenic in nude mice, it appears that stabilization of the 53K antigen by the SV40 large T protein is not required for tumorigenicity in these cell lines.

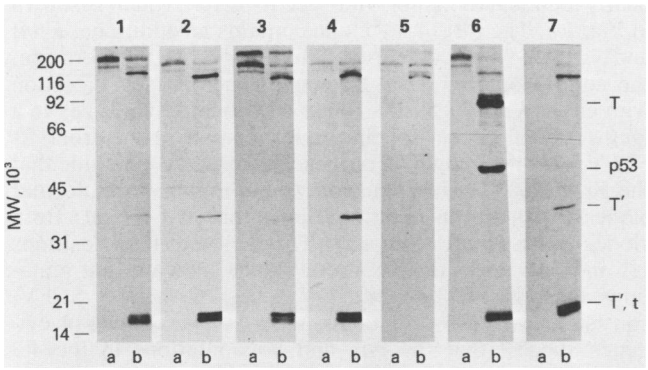

FIG. 1. Tumor antigens in tumor cell lines derived from nude mice. We labeled each cell line for 2.5 h with [^{35}S]methionine (1,000 Ci/mmol, 0.1 mCi/ml), extracted the labeled proteins, immunoprecipitated the extracts with hamster anti-SV40 tumor serum (b lanes) or with normal hamster serum (a lanes), and subjected the resulting precipitates to electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel (3, 5). Shown here is a fluorograph of that gel. Lanes: 1, NT51; 2, NT50; 3, NT49; 4, NT53; 5, untransformed 10T1/2 cells; 6, clone 98; 7, clone 32. Clone 98 is a wild-type SV40-transformed 10T1/2 line. Clone 32 is a line of 10T1/2 cells that has been transformed by F8*dl*. NT49, NT50, NT51, and NT53 are cell lines derived from tumors induced in nude mice by the F8*dl*-transformed cell lines clone 49, clone 50, clone 51, and clone 53, respectively. In these lines we see the truncated forms of T antigen (indicated by T') that are characteristic of F8*dl* transformants as well as the small t antigen.

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