

## Phenotype of Polyoma-Induced Hamster Tumor Cell Lines

MARK A. ISRAEL,<sup>1\*</sup> MALCOLM A. MARTIN,<sup>1</sup> TATSUO MIYAMURA,<sup>2†</sup> KENNETH K. TAKEMOTO,<sup>2</sup> DANIEL RIFKIN,<sup>3</sup> AND ROBERT POLLACK<sup>4</sup>

*DNA Recombinant Research Unit<sup>1</sup> and Virology Unit,<sup>2</sup> National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205; Department of Cell Biology, New York University Medical Center, New York, New York 10016<sup>3</sup>; and Sherman Fairchild Center for Life Sciences, Columbia University, New York, New York 10027<sup>4</sup>*

Cell lines from polyoma-induced hamster tumors exhibit a fully transformed phenotype despite the absence of the 105K (105,000-dalton) form of polyoma T-antigen.

Evaluation in tissue culture of cells which have undergone oncogenic transformation has contributed greatly to our understanding of malignancy. Cell lines which are available for such analysis have been derived from animal tumors or by the in vitro transformation of cells already in tissue culture. Some time ago, it was recognized that the altered growth properties displayed in tissue culture by papovavirus-transformed cells included a broad spectrum of phenotypes (21). A number of different assays have been employed to establish criteria by which such phenotypes could be quantitated and compared (20). Remarkably, cells which are "transformed" by some criteria may often not be "transformed" by other criteria (18, 19). For the most part, cell lines which have been systematically studied were established by the in vitro transformation of fibroblasts. Although it has generally been assumed that cell lines established in culture from virus-induced tumors possess a "fully transformed" phenotype, there is little experimental evidence to support this hypothesis. In this report, we have evaluated the phenotypes of a series of cell lines from hamster tumors induced by the inoculation of polyoma (PY) virus and PY DNA. Cell lines from such tumors, as well as some from in vitro-transformed PY hamster lines (7), generally do not contain the large form of PY tumor antigen (T-antigen; 105,000 daltons [105K]) but invariably do contain the middle (56K to 63K) and small (22K) PY T-antigens (10). Furthermore, PY DNA which has been interrupted in a portion of the genome which specifically encodes the large form of PY T-antigen (e.g., *EcoRI*-cleaved PY DNA which had been subsequently digested with *S<sub>1</sub>* nuclease [*EcoRI* + *S<sub>1</sub>* PY DNA]) induces a higher frequency of tumors after a shorter latency in newborn hamsters than PY DNA I

(9, 10). We were therefore curious to know not only the phenotype of such tumor cell lines, but also whether the absence of PY large T-antigen or the type of inducing viral agent (viz., virus, PY DNA I, or *EcoRI* + *S<sub>1</sub>* PY DNA) could be correlated with a specific phenotype.

The production of detectable plasminogen activator by tumor cells is an important biochemical correlate of oncogenic transformation (12). The presence of such proteases may be related to the cytoskeleton disruptions typical of transformed cells (13) and correlates well with the growth of such cells in agar (14, 15). We have examined several mass cultures from tumor tissue, as well as cell lines which had been cloned from these cultures by dilution in microtiter plates, for the presence of plasminogen activator, disruption of cytoskeleton organization, and growth without anchorage in agar (Tables 1, 2, and 3). All of the cell lines studied have been previously examined and found to contain PY DNA sequences (11; our unpublished data) and proteins specifically immunoprecipitated by anti-PY tumor serum (10; our unpublished data).

Though only one of the tumor cell lines, PYT-54, examined in this report contains the 105K PY T-antigen (10), all of the cell lines contain the middle and small forms of PY T-antigen. Interestingly, using a previously described Formalin fixation technique (6), we have recently been able to detect intense nuclear fluorescence in the nuclei of cells from all PY tumor cell lines (our unpublished data) evaluated with our standard anti-PY T-antigen tumor sera (10). Although we do not know which of the viral antigens are responsible for this nuclear fluorescence, it is unlikely that it is caused solely by truncated forms of PY large T-antigen, since we have been unable to identify such proteins in several of the cell lines evaluated in this paper.

We could detect no significant differences in any of the phenotypic characteristics examined

† Present address: Department of Enteroviruses, National Institute of Health, Musashi-Murayama, Tokyo, Japan.

TABLE 1. Production of plasminogen activator by PY-induced hamster tumor cell lines (17)

Original injection <sup>a</sup>	Cultures							
	Line	Mass			Line	Cloned		
		Plasminogen activator production <sup>b</sup>				Plasminogen activator production		
		Cells <sup>c</sup>	SFCM <sup>d</sup>	Total cell <sup>e</sup>		Cells	SFCM	Total cell
PY virus	PTL19	17	13	14	ND <sup>f</sup>			
	PTL45	9	16	50				
PY DNA I	PTL1	3	8	25	PTL1-1	6	16	28
					PTL28-1	17	20	80
PY DNA ( <i>EcoRI</i> + S <sub>1</sub> )	PTL10	6	16	30	PTL10-1	6	18	45
	PTL11	23	13	17	PTL11-F2	37	16	75

<sup>a</sup> Tumor cell lines were prepared and cloned as previously described (11). The inoculation of hamsters with PY virions, PY DNA I, or PY DNA cleaved with *EcoRI* and treated with S<sub>1</sub> nuclease has also been described (10).

<sup>b</sup> Plasminogen activator was assayed by the solution of <sup>125</sup>I-labeled fibrin (17). The numbers given are the percentage of available, trypsinizable counts digested in a given assay. Assays of plasminogen activator in cells, serum-free conditioned medium, and total cellular extracts (see below) of secondary hamster fibroblasts and rat neurotumors (8) yielded values of 11, 3, 7 and 96, 35, 60, respectively.

<sup>c</sup> Activity of cells plated on <sup>125</sup>I-labeled fibrin (cells). Cells were grown on <sup>125</sup>I-labeled fibrin in 2.5% dog serum for 16 h. The activity represents secreted plus surface-bound plasminogen activator.

<sup>d</sup> Activity of serum-free conditioned medium (SFCM). Cells were incubated in serum-free medium for 24 h, and then aliquots of this medium were assayed for plasminogen activator. The activity represents secreted plasminogen activator.

<sup>e</sup> Activity of total cell-associated plasminogen activator (total cell). Cultured cells were washed with phosphate-buffered saline, scraped, and pelleted. The cell pellet was disrupted with 0.1 M Tris (pH 8.1)-0.5% Triton. Each assay contained 2 μg of cell protein. Counts represent surface plus internal plasminogen activator.

<sup>f</sup> ND, Not determined.

TABLE 2. Actin organization in PY-induced hamster tumor cell lines

Original injection	Cultures			
	Mass		Cloned	
	Line	% Actin cables <sup>a</sup>	Line	% Actin cables
PY virus	PTL19	22 ± 5		
	PTL15	47		
PY DNA I	PTL1	58	PTL28-1	6
			PTL1-1	27 ± 2
PY DNA ( <i>EcoRI</i> + S <sub>1</sub> )	PTL10	18	PTL9-2	15
	PTL11	48	PTL11-F2	16 ± 5

<sup>a</sup> Cells were grown and fixed as previously described (6), but stained serially with rabbit anti-actin immunoglobulin G (1:20) and goat anti-rabbit immunoglobulin G-rhodamine (1:10) (Cappel Labs) and examined for rhodamine epifluorescence with a 63× lens. The arbitrary criterion for positive actin organization was taken to be the detection of at least two stress fibers or cables running the length of the cell under the nucleus, while focusing on the edge of the cell. Such cables lie close to the adherent surface of the cell. By this criterion, 75 to 100% of normal cells in fibroblast cultures from a wide variety of species and tissues are positive (14).

between cell lines established from tumors induced by PY virus and those induced by PY DNA I or *EcoRI* + S<sub>1</sub> PY DNA. Although every tumor line examined clearly possessed the fully transformed phenotype characterized by active

plasminogen activator production, dissociated actin cables, and avid anchorage-independent growth, the variation among several lines induced by either PY virus or PY DNA was as great as the variation between these two types of cells. Furthermore, in almost every case examined, the cloned lines expressed these various phenotypic characteristics more intensely than did the mass cultures from which these lines were derived. Since each of the mass cultures had been passaged in tissue culture before evaluation, this finding suggests that the cloning of cell lines by dilution techniques may select for a more extreme phenotype than that observed upon examination of the total tumor cell population.

To examine further the growth properties of these cell lines, we examined their ability to form tumors in weanling hamsters. As indicated by the number of cells required to produce a tumor in 50% of the animals inoculated (TPD<sub>50</sub>), all of the lines examined were highly oncogenic in animals (Table 4). Furthermore, the oncogenic potential of these lines was not significantly different whether the line had been established from a hamster tumor induced by virus (PYT-54), PY DNA I (PTL 1-1), or by *EcoRI* + S<sub>1</sub> PY DNA (PTL 9-2).

TABLE 3. Growth in agar of PY-induced hamster tumor cell lines

Original injection	Cultures					
	Mass			Cloned		
	Line	% PE in agar in serum <sup>a</sup>		Line	% PE in agar in serum	
		FCS	DOG		FCS	DOG
PY virus	PTL19	30	24			
	PTL15	15	38			
PY DNA I				PTL1-1	1.6	1.4
				PTL28-1	27	34
PY DNA				PTL10-1	3.2	7.6
(EcoRI				PTL11-F2	24	31
+ S <sub>1</sub> )				PTL9-2	2.3	22

<sup>a</sup> Percent plating efficiency (PE) in the absence of anchorage was assayed by inoculating  $10^5$  to  $10^3$  cells onto hard agarose, in a suspension of soft agarose. Colonies were scored at 3 weeks. The minimum sized colony scored was 0.2-mm diameter, containing ca. 1,000 cells. Sera used were either 10% fetal calf serum (FCS) or 5% dog serum plus 5% FCS (DOG). The latter serum enhances agar growth for some lines secreting plasminogen activator. Dog serum plasminogen is more easily activated than FCS plasminogen. Normal fibroblast PE is 0.01% in either serum.

We have entertained a number of hypotheses to explain the enhanced tumorigenic potential of PY DNA which has been disrupted by enzymatic cleavage in the distal portion of the early region (9, 10). Amongst these was the possibility that cells transformed by such altered DNA substrates would be incapable of synthesizing the PY tumor-specific transplantation antigen (TSTA) and thereby escape immunosurveillance. Hoping to correlate the enhanced tumorigenic potential of such cleaved PY DNA with the TSTA activity present in cell lines from such tumors, we compared the TSTA present in cell lines established from tumors induced by PY virus (PYT-54), PY DNA I (PTL 1-1), and EcoRI + S<sub>1</sub> PY DNA (PTL 9-2). Table 4 shows the rejection index (the ratio of the TPD<sub>50</sub> of a particular cell line in animals immunized by inoculation with PY virus to the TPD<sub>50</sub> of that cell line in unimmunized animals) for each of the lines examined. No detectable difference in the TSTA activity of cell lines established from tumors induced by either PY virus, PY DNA I, or PY DNA interrupted in the distal portion of the early region could be detected, suggesting that the enhanced tumorigenicity observed with cleaved PY DNA was not due to the absence of TSTA in cells transformed by these DNAs. Although PYT-54 contains the large, middle, and small forms of the PY T-antigens, PTL 1-1 and

TABLE 4. Transplantation immunity in Syrian hamsters to PY-induced hamster tumor cell lines<sup>a</sup>

Tumor cell line	Immunization	TPD <sub>50</sub>	Rejection index
PYT-54	—	10 <sup>4.3</sup>	16
	PY	10 <sup>5.5</sup>	
PTL1-1	—	10 <sup>2.5</sup>	16
	PY	10 <sup>3.7</sup>	
PTL9-2	—	10 <sup>3.7</sup>	12
	PY	10 <sup>4.8</sup>	

<sup>a</sup> Eight-week-old male Syrian golden hamsters were immunized by subcutaneous inoculation of PY virus ( $10^7$  PFU/animal). Five days later, the animals were injected with a second dose of PY virus. One week after the second immunization, the animals were inoculated subcutaneously with serial 10-fold dilutions of tumor cells (five animals per dilution) and observed for 2 months. PYT-54 is a cell line from a hamster tumor initially induced by inoculation of PY virions. It contains the large (105K), middle (56K), and small (22K) forms of PY T-antigen (10). PTL1-1 is a cell line from a tumor induced by PY DNA I, and PTL9-2 is from a tumor induced by PY DNA treated with EcoRI and S<sub>1</sub> nuclease. These cell lines contain the 56K and 22K forms of PY T-antigen but do not contain the 105K PY large T-antigen (10). The TPD<sub>50</sub> was calculated by the Reed and Muench method (16). The rejection index is the ratio of the TPD<sub>50</sub> of a particular cell line in animals immunized by PY virus to the TPD<sub>50</sub> of that same cell line in unimmunized animals.

PTL 9-2 do not contain the full-sized PY large T-antigen (10; our unpublished data). Thus, full-sized PY large T-antigen is not critical in determining the transplantation immunity of PY transformed cells.

The data presented in this study would suggest that although several patterns of alterations in cellular growth properties may be observed in in vitro-transformed cells (3, 18-20), tumor cell lines display a remarkable similarity in their expression of the transformed phenotype. It is unknown whether the "fully transformed" phenotype which we observe in these tumor cell lines is a "final common cell type" which arose through progressive steps in a pathway along which the transformed state develops or if it is one of several phenotypes which occur after oncogenic transformation and is selected for on the basis of its unique ability to lead to tumor formation. Our finding that expression of various phenotypic characteristics of transformation were less intense in mass cultures of tumor tissue than in cell lines cloned from these cultures suggests that virus-induced tumors are not biologically homogeneous populations. If such tumors consist of several subpopulations, it would be important to know the phenotype of these cells and how they interact within the tumor tissue.

Expression of the fully transformed phenotype in tumor cells from PY-induced tumors lacking the large T-antigen confirms and extends previous genetic studies which indicated that the PY a-gene function was not required for maintenance of transformation in cells transformed in vitro (1-5, 22). Furthermore, these studies clearly exclude a function for the distal part of PY large T-antigen in the maintenance of any aspect of the in vitro-transformed state. The contributions made by the PY small T-, middle T-, and proximal fragments of large T-antigens in oncogenic transformation remain to be sorted out by the appropriate genetic (6) and biochemical tests.

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