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Genetics

Tumorigenicity and its suppression in cybrids of mouse and Chinese hamster cell lines

(genetics/cell hybrids/cytoplasmic inheritance/cell transformation/cancer)

A. NEIL HOWELL* AND RUTH SAGER*†‡

* Sidney Farber Cancer Institute, Department of Molecular Genetics, 44 Binney St., Boston, Massachusetts 02115; and ‡ Department of Microbiology, Harvard Medical School, Boston, Massachusetts 02115

Contributed by Ruth Sager, December 27, 1977

ABSTRACT **The effect of cytoplasm upon the expression of tumorigenicity was examined with a pair of mouse and a pair of Chinese hamster cell lines in intraspecies cybrids formed by reciprocal fusions between either tumorigenic or nontumorigenic cells and cytoplasms derived from them. With the mouse cells, 3T3 and the simian virus 40-transformed line SVT2, the cybrid clones were tumorigenic when SVT2 cells were fused with 3T3 cytoplasts, but not in the reciprocal fusion. With the hamster cells, CHEF/18 and the spontaneous transformant CHEF/16, however, tumorigenicity was partially suppressed in cybrid clones formed by fusion of tumorigenic CHEF/16 cells with CHEF/18 cytoplasts; cybrids were nontumorigenic in the reciprocal fusion. Thus, cybrid analysis has shown that tumorigenicity is not cytoplasmically transmitted in these two cell pairs, but suppression of tumor-forming ability may be cytoplasmically transmitted in the hamster cybrids.**

The existence of cytoplasmic genes in eukaryotic cells was established by the discovery of organelle DNAs in chloroplasts (1, 2) and in mitochondria (3, 4) and by the recovery of organelle mutants which led to genetic mapping of chloroplast (5, 6), and subsequently of mitochondrial (7, 8), genomes. Long before these discoveries, Otto Warburg proposed, on the basis of his observations of the greatly increased rate of aerobic glycolysis in various tumors, that a defect in the respiratory apparatus lay at the molecular basis of cancer (9).

It has now become possible to test one implication of Warburg's hypothesis directly, namely, whether the respiratory defects associated with tumorigenicity result from changes in mitochondrial DNA or in any other cytoplasmic genes. New methods of parasexual genetic analysis with mammalian cells in culture (10) include not only cell fusion to produce cell hybrids (11), but also fusion of a cell with an enucleated cell (cytoplast) to form a cybrid (12) and fusion of a cytoplast with a karyoplast to form a reconstituted cell (13). Cell hybrids produced by fusion of pairs of cells, one tumorigenic and the other not, provide material to assess the expression of tumorigenicity in the hybrids (14–20).§

In this study we examine two pairs of cell lines: (*i*) a pair of mouse fibroblasts, the nontumorigenic BALB 3T3 (clone A31) and the BALB embryo cell line transformed by Simian virus 40, SVT-2 (21); and (*ii*) a pair of Chinese hamster fibroblast cell lines, CHEF/18-1, which is nontumorigenic, and CHEF/16-2, which forms tumors in *nude* mice (20). In concurrent studies, we have examined the expression of tumorigenicity in hamster (20) and mouse§ hybrids. In both pairs, the transformed phenotype and tumorigenicity were suppressed in the hybrids, but reappeared in a small subset of hybrid cells that formed tumors in *nude* mice (see *Discussion*).

We report here a study of cybrid analysis directed towards the same question: identification of genetic components involved in the expression and suppression of tumor-forming ability. Cybrids provide material with which to distinguish whether the expression of tumorigenicity is determined by nuclear or by cytoplasmic genes and also to examine transient effects transmitted through the cytoplasm but subsequently lost.

MATERIALS AND METHODS

Cells and Media. Chinese hamster cell lines were grown with α-minimal essential medium (Kansas City Biological Co.) plus 10% fetal calf serum (Flow Lab. or Microbiological Associates) plus antibiotics (100 units of penicillin per ml, 100 μg of streptomycin per ml) in a humidified incubator with 5% CO_2. In some experiments, noted in the text, α-HG (α medium with 4.5 mg of glucose per ml) was used. Mouse cell lines were grown in Dulbecco's modified Eagle's medium containing 4.5 mg of glucose per ml and no pyruvate (Flow Lab.), plus 10% calf serum (Colorado Serum Co.) plus antibiotics at 37° in a humidified incubator with 10% CO_2.

In the hybrid studies, the fusion products were recovered by the hypoxanthine/aminopterin/thymidine (HAT) selection system (22). For cybrid selection, one additionally requires a cytoplasmic genetic marker; we used chloramphenicol (CAP) resistance. The tumorigenic potential of cytoplasts from tumor cells was examined by fusion of nontumorigenic cells carrying a recessive nuclear mutation conferring resistance to 6-thioguanine (SGua) or bromodeoxyuridine (BrdUrd) with cytoplasts from tumorigenic cells carrying the CAP resistance mutation. The cybrids were selectively recovered by plating the mixture in medium containing CAP and BrdUrd or SGua. In the reciprocal cross, to examine the effect of cytoplasm from nontumorigenic cells on the expression of tumorigenicity, CAP-resistant cytoplasts from nontumorigenic cells were fused with tumorigenic cells.

Cybrid Production. Cytoplasts were prepared by the procedure of Veomett *et al.* (23) with slight modifications for some cell lines. Cells were seeded to form a confluent monolayer, incubated overnight in collagen-coated flasks (25 cm^2), and then centrifuged in fresh growth medium containing 10 μg of cytochalasin B per ml (Aldrich Chem. Co.) at 37° in a Sorvall RC-5 centrifuge. Hamster cells were enucleated (80–90%) at

Abbreviations: CAP, chloramphenicol; HAT, hypoxanthine/aminopterin/thymidine; SGua, 6-thioguanine.

† To whom reprint requests should be addressed.

§ N. Howell and R. Sager, unpublished data.

Table 1. Properties of mouse parents and cybrids formed by fusion of SVT2 CAP^R cytoplasts and 3T3 BrdUrd^R cells

Cell line	Morphology	Plating efficiency, % 10% serum	1% serum	Methylcellulose	Tumorigenicity Tumor	Inoculum	Time, weeks	No. of chromosomes*
Parents								
SVT2 CAP^R (280-7)	SVT2	41	9.0	45	2/2	10^6	4	39 ± 2.4 (12)
3T3 BrdUrd^R (257-3)	3T3	51	0.02	6×10^{-4}	0/6	2×10^7	28	65 ± 1.6 (24)
SVT2 (SGua^R, CAP^R)†	SVT2	75	45	66	2/2	10^6	3	39 ± 1.0 (12)
Cybrids								
MC 104-4†	3T3	35	<0.02	2×10^{-4}	0/2	10^7	34	64 ± 2.2 (26)
MC 106-2†	3T3	48	<0.01	5×10^{-4}	0/2	10^7	34	63 ± 2.8 (23)
MC 106-5†	3T3	48	3.5	4×10^{-4}	0/2	10^7	34	63 ± 2.8 (30)
MC 120-5	3T3	62	<0.02	$<10^{-4}$	—	—	—	65 ± 1.7 (9)
MC 120-10	3T3	53	<0.01	$<2 \times 10^{-4}$	0/2	6×10^6	33	64 ± 2.0 (11)
MC 120-11	3T3	67	<0.01	$<2 \times 10^{-4}$	0/2	1.2×10^7	34	
MC 120-12	3T3	58	<0.01	$<2 \times 10^{-4}$	0/2	10^7	34	61 ± 2.3 (22)
MC 120-15	3T3	59	<0.02	$<2 \times 10^{-4}$	0/2	10^7	34	

* Means ±SD are given. Number in parentheses is the number of cells counted. Tetraploids (0–4%) omitted from range.
† Cybrids designated 104 and 106 were formed with SVT2 (CAP^R) 280-7 as the cytoplast parent; for the 120 series, SVT2 (CAP^R, SGua^R) 107-6-4 cytoplasts were used.

9000 rpm for 20 min. SVT2 cells were enucleated (95–99%) at 8500 rpm for 15 min, 3T3 cells (70–80%) at 10,000 rpm for 40 min. After enucleation the cytoplasts were washed with complete medium and incubated for 1 hr. For cell fusion, cytoplasts were washed with serum-free medium at 4°; 1000 hemagglutinating units/0.25 ml of inactivated Sendai virus (Microbiological Associates) was added for 20 min at 4°; then the cell suspension was added and the mixture was incubated for 20 min at 4° and then for 60 min at 37°. After the cultures were washed with complete medium plus serum, they were incubated overnight, then trypsinized and replated in complete medium with 50 μg of CAP at 10^4–10^5 cells/100-mm petri dish. When BrdUrd (50 μg/ml) was used for selection it was added at this time; SGua (20 μg/ml) was added after 4–6 days in some crosses and after 8–10 days in others. Macroscopic cybrid colonies were isolated with cloning rings after 2–3 weeks of growth and transferred to 35-mm dishes containing selective medium.

Assays. Plating efficiencies were measured by plating 200–2000 cells per 100-mm dish. Macroscopic colonies were counted after 10–14 days. Anchorage dependence was assayed by plating serial dilutions (10^2–10^5 cells) in 60-mm petri dishes containing a base of 0.6% agar in growth medium, overlaid with a 5-ml suspension of cells in medium containing methylcellulose (1.3%). Assay plates were re-fed at weekly intervals and macroscopic colonies scored after 4–8 weeks. Tumorigenicity was assayed in *nude* mice (24). Cells were injected subcutaneously at 10^6–2×10^7 cells per site; mice were examined weekly and considered positive when growths reached 0.5 cm in diameter; mice without tumors were kept at least 12 weeks before they were scored as negative. Chromosome counts were performed as described (20) except that counts of mouse chromosomes were made from photographs. Cell lines were checked for mycoplasma contamination as described (25).

RESULTS

Origin and Properties of Parental Cell Lines. In the SVT2 mouse cell line, a HAT-sensitive, SGua-resistant mutant was isolated after treatment with nitrosoguanidine (4 μM, 1 hr, 10% survival). The mutant resembles the parental cell line in properties relevant to this study (Table 1). Several HAT-sensitive, BrdUrd-resistant clones were recovered by a two-step selection procedure: plating mutagenized cells first on medium with 3 μg of BrdUrd per ml and subsequently on medium with 50 μg of BrdUrd per ml. The subclone selected for this study (288-7) resembles the SVT2 parent in its high plating efficiency on complete medium and in semisolid medium, but differs from it in a rather low plating efficiency in medium with 1% serum, and in a modal chromosome number of 57–59 instead of the parental number of 40 (Table 2).

CAP-resistant SVT2 mutants were recovered after 10^5 cells

Table 2. Properties of mouse parents and cybrids formed by fusion of 3T3 CAP^R cytoplasts and SVT2 BrdUrd^R cells

Cell line	Morphology	Plating efficiency, % 10% serum	1% serum	Methylcellulose	Tumorigenicity Tumor	Inoculum	Time, weeks	No. of chromosomes*
Parents								
SVT2 BrdUrd^R (288-7)	SVT2	24	1.4	22	2/2	10^6	4	56 ± 3.7 (25)
3T3 CAP^R (284-14)	3T3	22	0.02	3×10^{-4}	0/5	8×10^6	27	64 ± 2.8 (28)
Cybrids								
MC 121-1	SVT2	40	0.8	9.6	2/2	10^6	6	57 ± 4.6 (27)
MC 121-2	SVT2	29	0.1	3.8	—	—	—	—
MC 121-4	SVT2	16	0.4	19	2/2	10^6	6	56 ± 2.1 (19)
MC 124-33	SVT2	43	0.2	46	2/2	10^6	7	56 ± 6.0 (25)
MC 124-34	SVT2	42	9.3	26	2/2	10^6	6	53 ± 1.8 (22)
MC 124-37	SVT2	47	0.9	20	2/2	10^6	12	57 ± 1.8 (29)

* Means ±SD are given. Number in parentheses is the number of cells counted. Tetraploids (0–4%) omitted from range.

Table 3. Properties of hamster parents and cybrids formed by fusion of 16-2 CAP^R cytoplasts and 18-1 SGua^R cells

Cell line	Morphology	Plating efficiency, % 10% serum	3% serum	Methylcellulose	Tumorigenicity Tumor	Inoculum	Time, weeks	No. of chromosomes*
Parents								
16-2 CAP^R (213-21-3)	16-2	80	10	35	2/2	4×10^6	6–10	21 (30) 20–22
18-1 SGua^R (205-30)	18-1	65	0.03	$<2 \times 10^{-4}$	0/3	4×10^6	16	22 (46) 21–24
Cybrids								
HC 111-2	18-1	38	0.01	$<2 \times 10^{-4}$	0/4	4×10^6	33	22 (27) 19–23
HC 111-13	18-1	14	0.02	$<2 \times 10^{-4}$	0/2	4×10^6	9†	22 (25)
					0/2	4×10^6	33	20–24
HC 111-14	18-1	31	0.02	$<2 \times 10^{-4}$	0/2	4×10^6	9†	22 (27)
					0/2	4×10^6	33	19–23
HC 119-2	18-1	23	0.02	$<2 \times 10^{-4}$	0/4	4×10^6	33	22 (26) 20–23

* Mode and range are given. Number in parentheses is number of cells counted. Tetraploids (0–4%) omitted from range.
† Mice died.

were plated per 100-mm dish in modified Eagle's medium plus 50 μg of CAP per ml. Clones appeared at a frequency of about 10^{-5} in 2–3 weeks. Resistance was stable after prolonged passage in the absence of the drug, but cultures were routinely maintained in medium plus CAP. Mutants were indistinguishable from the parental SVT2 in the properties examined in this study other than CAP resistance (Table 1). CAP-resistant mutants of line 3T3 were recovered after selection in α-HG medium plus 50 μg of CAP per ml, but are capable of growth indefinitely in either α-HG or modified Eagle's medium plus 50 μg of CAP per ml. The CAP resistance of these mutants is stable in the absence of the drug. Modified Eagle's medium plus

Table 4. Properties of hamster parents and cybrids formed by fusion of 18-1 CAP^R cytoplasts with 16-2 BrdUrd^R cells

Cell line	Morphology*	Plating efficiency, % CAP	10% serum	3% serum	Methylcellulose	Tumors in nude mice† Tumor	Inoculum	Time, weeks	No. of chromosomes‡
16-2 BrdU^R						2/2	2×10^6	6–8	
204-BU50	16-2	$<2 \times 10^{-3}$	34	6.8	26	4/4	4×10^6	6–8	22 (50) 20–22
18-1{CAP^R}; SGua^R									
294-7	18-1	12	9	<0.02	$<10^{-3}$	0/2	4×10^6	22	22 (26) 20–24
HC 163-3-1	INT	11	20	0.03	8.2	0/2	2×10^6	23	22 (25)
						0/2	4×10^6	6	20–22
-2	16-2	7	14	1.8	3.8	0/2	2×10^6	23	22 (20)
						0/2	4×10^6	6	19–23
-3	INT	15	20	<0.04	6.6	0/2	2×10^6	23	21 (25) 19–26
-4	INT	4	11	1.1	0.7	2/2	2×10^6	11	22 (32) 20–24
-5	INT	32	59	1.5	41	0/2	2×10^6	23	21 (50) 20–22
-6	INT	5	26	0.3	6.3	0/2	2×10^6	23	22 (25) 18–24
-8	INT	7	11	0.5	0.2	0/2	4×10^6	6	21 (25) 19–22
-9	INT	10	18	0.6	14	0/2	2×10^6	23	
						0/2	4×10^6	6	
-10	INT	29	49	0.2	3.7	1/2	2×10^6	16	21 (46) 21–22
-11	16-2	34	53	6.1	37	0/2	2×10^6	23	21,22 (30) 19–22
-12	INT	27	29	0.2	5	0/2	2×10^6	23	20 (25) 18–22

* INT, intermediate.
† Mice are scored as positive when the tumor reaches 0.5 cm. Slowly growing regressing nodules of 1–3 mm in diameter have appeared on mice injected with cybrids 6, 11, and 12. Other cybrids have produced no sign of growth.
‡ Mode and range are given. Number in parentheses is number of cells counted. Tetraploids (0–4%) omitted from range.

CAP is used routinely for cybrid selection since CAP-sensitive cells make fewer doublings before death in this medium plus CAP than in alpha HG plus CAP. The procedure used to isolate HAT-sensitive, BrdUrd-resistant 3T3 mutant used in these studies (Table 1) will be described elsewhere.§

The origin and properties of the two CHEF mutant cell lines used for hybrid selection have been described in detail elsewhere (20). Relevant data are summarized in Tables 3 and 4. The SGua-resistant, nontumorigenic mutant 205-30 is derived from CHEF/18-1; the BrdUrd-resistant, tumorigenic mutant 204-50 is derived from CHEF/16-2.

CAP-resistant mutants have been isolated from 205-30 and from CHEF/16-2 after some difficulty. CAP-resistant clones can be recovered at a frequency of about 10^{-5} when 16-2 cells are inoculated into α medium plus 50 μg of CAP per ml. The recovered clones that are CAP-resistant can be passaged indefinitely in NCTC 135 medium plus 50 μg of CAP per ml containing 4.5 mg of glucose per ml and 10% fetal calf serum, but not in α plus CAP. All cybrid experiments and testing for CAP resistance were done in α plus CAP, but long-term passaging was done in the NCTC medium. These CAP-resistant mutant clones derived from CHEF/16-2 plate well in 3% serum and in semisolid medium and are tumorigenic in *nude* mice (Table 3).

CAP-resistant mutant clones were derived from 205-30 by mutagenizing cells with nitrosoguanidine and selecting in a 1:1 mixture of α and NCTC 135 containing 50 μg of CAP per ml, 4.5 mg of glucose per ml, and 10% fetal calf serum. One CAP-resistant clone (294-7) was chosen for these studies (Table 4). Like its parental cell line, this mutant does not clone in 3% serum, is anchorage dependent, and does not form tumors in *nude* mice. All four CHEF mutants used in this study have a narrow distribution of chromosomes, with a mode of 22 (20).

The results in Table 5 are a summary of the cybrid crosses carried out. All the CAP mutants transmit their drug resistance from cytoplasts to progeny cybrid cells. Further evidence of cytoplasmic transmission comes from chromosome counts, which are indistinguishable from those of the nucleated parent (Tables 1–4), and from the fact that the frequency of spontaneous mutations to CAP resistance is 10^{-5} or below in these cell lines.

Cybrids of Mouse SVT2 and 3T3 Cell Lines. The results of fusion between cytoplasts of the tumorigenic SVT2 line and 3T3 TK^- cells are shown in Table 1 for a set of eight cybrids of independent origin. All eight clones show a standard 3T3 colony morphology, are dependent on anchorage for growth, and are nontumorigenic under our conditions of assay. All but one clone (106-5-3) have retained the stringent serum requirement of the 3T3 BrdUrd-resistant nucleated parent. Another 12 cybrids of this type have been tested for serum and anchorage requirements; all closely resemble the 3T3 parent. If there were a transient expression of transformation by these cybrids when they were first formed, it would be difficult to detect under the conditions of cybrid selection.

In the reciprocal cybrids shown in Table 2, there is little if any evidence of suppression. Two of the clones show slightly reduced cloning ability in methylcellulose, but morphology and tumor-forming ability resemble the tumorigenic parent.

Cybrids of Chinese Hamster 16-2 and 18-1 Cell Lines. The properties of cybrids formed from fusion of 16-2 CAP-resistant cytoplasts and 18-1 SGua-resistant cells are shown in Table 3. All cybrids had the characteristic 18-1 cell and colony morphology (20). Like the 18-1 parent, the cybrids did not clone appreciably in medium containing 3% serum, did not have an enhanced cloning frequency in semisolid medium, and were nontumorigenic. Thus, these cybrids show no evidence of cytoplasmic transmission of the transformed phenotype.

Table 5. Cytoplasmic transmission of CAP resistance in mouse and hamster cybrids

Parent			
Cytoplast	Nuclear	Conditions*	Cybrids†/10^5 cells plated‡
		Mouse–mouse cybrids	
3T3 CAP^R 284-14	3T3 $BrdUrd^R$ 257-3	−Virus	4
		+Virus	125
SVT2 CAP^R 280-7	SVT2 $BrdUrd^R$ 288-7	+Virus§	0
		−Virus	0, 0, 0
		+Virus	9, 38, 131
SVT2 CAP^R 280-7	3T3 $BrdUrd^R$ 257-3	+Virus§	3
		−Virus	0, 0, 3
		+Virus	42, 67, 485
		Hamster–hamster cybrids	
16-2 CAP^R 213-21	16-2 $BrdUrd^R$ 204-50	+Virus§	0
		−Virus	0
		+Virus	29
16-2 CAP^R 213-21	18-1 $SGua^R$ 205-30	−Virus	0, 0
		+Virus	38, 78
18-1 TG-R CAP^R 294-7	16-2 $BrdUrd^R$ 204-50	+Virus§	0
		−Virus	0
		+Virus	12

* Cells plated onto medium containing 50 μg of CAP per ml at 1–3 × 10^4 per petri dish; BrdUrd added first day, SGua added 3–6 days after plating.
† See Tables 2–5 for chromosome counts.
‡ Numbers represent independent experiments.
§ Not enucleated.

The last set of cybrids to be presented here was formed by fusion of cytoplasts of the nontumorigenic CAP and SGua-resistant 18-1 mutant (294-7) with the tumorigenic 16-2 cells. The characteristics of the parents and cybrids from this experiment are presented in Table 4. In marked contrast to the other cybrid series described here, these cybrids do *not* have the phenotype of the nucleated parent; rather, the cytoplasm of the nontumorigenic parent suppresses transformation in the cybrids. The parental 16-2 cells (i.e., 204-BU50) have a distinctive colony morphology (20) in which the cells form tight, multilayered "bundles." Cybrid colonies, in contrast, generally form colonies of a more irregular shape with less piling up; this morphology is very similar to that of the nontumorigenic cytoplast parent, but the cybrid colonies differ from it in having many loosely attached cells on top of the colony. Two of the cybrids, however, gave rise to colonies with the morphology of the 16-2 parent. With the exception of cybrid 11, all the cybrid lines were reduced in their efficiency of plating in medium with 3% serum compared to the nucleated parent. Those clones that did arise in low serum had a morphology similar to the 16-2 parent. All the cybrids except HC 163-5 and HC 163-11 cloned with a reduced efficiency in methylcellulose. The results of tumorigenicity assays show that 2 of the 11 cybrids tested had tumors after 11 and 16 weeks, and the other 9 cybrids were still negative after 23 weeks. All testing, both in culture and in *nude* mice, was performed with populations that had undergone 20–30 doublings after fusion.

DISCUSSION

The experiments presented in this paper were designed to look for tumorigenicity or its suppression in reciprocal cybrids formed by fusions between pairs of tumorigenic and nontumorigenic cells, one enucleated and the other not. We found no evidence that cytoplasm coming from the tumorigenic parent could induce tumorigenicity or significantly alter any growth parameters we examined in either the mouse or the Chinese hamster cells. Further, we found no consistent effect of cytoplasm from normal (3T3) mouse cells upon tumorigenicity of SVT2 cells. In contrast, we found suppression in hamster cybrids by cytoplasm from the nontumorigenic cells fused with their tumorigenic nucleated counterparts, as assessed by cell culture parameters of transformation and by rate and extent of tumor formation in *nude* mice.

In concurrent studies with the same pairs of hamster (20) and mouse[§] cell lines, we have found strong suppression of tumorigenicity in cell hybrids, confirming and extending the studies by Harris, Klein, and coworkers (reviewed in refs. 15, 16 and 18). In those hybrids that did induce tumors in our studies, the subclones of tumor origin differed substantially from the original hybrid clones in their increased tumorigenicity and anchorage independence. Thus, subsets of cells with increased tumor potential were selected during growth in *nude* mice. Chromosome loss was not extensive in the tumor subclones from the mouse hybrids, whereas in the hamster, the tetraploidy of the hybrids was reduced down to the diploid range in all but one tumor subclone. This reduction provides us with unique material for identification of particular chromosomes associated with tumor formation or its suppression and for application to mapping, to studies of chromosome elimination mechanisms, and possibly to recombination.

Jonasson *et al.* (18) have reported that no particular chromosome could be correlated with tumorigenicity in a series of hybrids formed between malignant and diploid mouse cells. They proposed the role of an "extrachromosomal element." In a subsequent paper, in which malignant mouse cells were fused with normal human cells, Jonasson and Harris (19) found suppression of tumorigenicity even after loss of all recognizable human chromosome material and proposed the centrosome as the cytoplasmic component of human origin responsible for suppression.

With respect to the genetic basis of cytoplasmic suppression, we have considered: permanent cytoplasmic genes such as those in mitochondria; nuclear gene products with very long half-lives, e.g., structural components (26), cell membranes (27), or centrioles (28); and epigenetic effects of cytoplasmic components on nuclear gene expression. The fact that the cybrids have maintained their suppressed phenotypes for 20–30 doublings probably rules out the role of well-understood gene products such as mRNAs and proteins.

Experiments at hand for distinguishing among these possibilities include many generations of subculture followed by testing at intervals to look for loss of suppression and use of the cybrids themselves as donors in second generation cybrid and hybrid crosses. Stability of the suppressed phenotype and transmission in successive cybrid crosses would support the role of a permanent cytoplasmic gene in suppression. If tumor cell mitochondria are defective, as postulated by Warburg (9), then suppression could result from the introduction of mitochondria from normal cells into cybrids.

Suppression may be viewed as an allelic alternative to tumorigenicity, or it may be an independent phenomenon operating on a different pathway from those that lead towards the transformed state. The significance of suppression, whether nuclear or cytoplasmic, lies in its role as a cellular defense mechanism against carcinogenesis. Thus, the identification of genes and gene products responsible for suppression could provide the basis for a new approach to cancer chemotherapy.

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