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Loss of tumor-suppressive function during chemically induced neoplastic progression of Syrian hamster embryo cells

(carcinogens/oncogenes/immortality/Ha-*ras*/anti-oncogene)

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ABSTRACT **Cell hybrids between normal, early-passage Syrian hamster embryo cells and a highly tumorigenic, chemically transformed hamster cell line, BP6T, were formed, selected, and analyzed. Tumorigenicity and anchorage-independent growth were suppressed in the hybrid cells compared to the tumorigenic BP6T cells. These two phenotypes segregated coordinately in these cells. To determine at what stage in the neoplastic process this tumor-suppressive function was lost, two chemically induced immortal cell lines were examined at different passages for the ability to suppress the tumorigenic phenotype of BP6T cells following hybridization. Hybrids of BP6T cells with the immortal, nontumorigenic cell lines at early passages were suppressed for tumorigenicity and anchorage-independent growth. This tumor-suppressive ability was reduced in the same cells at later passages and in some cases nearly completely lost, prior to the neoplastic transformation of the immortal cell lines. Subclones of the cell lines were heterogeneous in their ability to suppress tumorigenicity in cell hybrids; some clones retained the tumor-suppressive ability and others lost this function. The susceptibility to neoplastic transformation of these cells following DNA transfection with the viral *ras* oncogene or BP6T DNA inversely correlated with the tumor-suppressive ability of the cells. These results suggest that chemically induced neoplastic progression of Syrian hamster embryo cells involves at least three steps: (*i*) induction of immortality, (*ii*) activation of a transforming oncogene, and (*iii*) loss of a tumor-suppressive function.**

The conversion of a normal cell into a malignant cell is recognized as a multistep process (1–3); however, the number of genetic changes involved is not known. A major advance in our understanding was the discovery of oncogenes that are capable of transforming immortal cells as well as normal, primary rodent cells when certain combinations of oncogenes are transfected into the cells (4–7).

These experiments indicate that at least two cooperating, apparently dominantly acting, oncogenes are required for neoplastic transformation of normal, diploid cells. It has been proposed that one oncogene is involved in the immortalization process and the second in the expression of various transformed phenotypes, such as focus formation or anchorage-independent growth (3, 4, 7). However, certain observations suggest that changes in addition to these two steps are also needed. One of the most compelling lines of evidence comes from experiments involving hybridization of normal and malignant cells. Many, but not all, of these experiments indicate that tumorigenicity is a recessive trait (8–14). A major paradox in cancer biology, therefore, exists: DNA transfection experiments have identified dominantly acting cancer genes (oncogenes), whereas cell hybridization experiments suggest that tumorigenicity is recessive in nature. These seemingly disparate results were recently demonstrated in parallel with the same cells. Craig and Sager have shown that the *ras* oncogene can transform Chinese hamster CHEF-18 cells following transfection but hybrids between oncogene-transformed cells and the nontransformed CHEF-18 cells were nontransformed (13). These observations suggested to these authors that a suppressive function, possibly an anti-oncogene, is operative in the nontransformed cells and has to be lost for the expression of transformation (13). Other lines of evidence are also consistent with this hypothesis. Tumors arising from the neoplastic transformation of Syrian hamster embryo (SHE) cells by v-Ha-*ras* plus v-*myc* oncogenes have a nonrandom loss of hamster chromosome 15, suggesting that a suppressor gene is lost for the expression of tumorigenicity (15). If immortalization and transforming events were sufficient for neoplastic transformation, then it would be expected that all immortal cell lines would be equally sensitive to the transforming effects of certain oncogenes, such as *ras*, and to chemicals that induce mutations in these genes. However, the frequency of transformation of rat cell lines to Kirsten murine sarcoma virus and Abelson murine leukemia virus varies even though the infection of the different cell lines is not different (16). Also, different clones of an established Syrian hamster cell line transformed by v-Ha-*ras* oncogene vary in their frequency of transformation and tumor latency period (17). Some immortalized cells are refractory to *ras* transformation (18, 19).

Carcinogen-induced transformation of cells in culture is also a multistep process and an early step involves immortalization of the cells (1, 2, 6, 20). Chemically induced immortal cells then require a number of subcultures or passages before the cells undergo final neoplastic transformation. Different immortal cell lines vary considerably in their rate of subsequent changes. For example, the rate of progression to anchorage-independent growth varies by three orders of magnitude between different cell lines (2). Immortal cells also vary significantly in their response to carcinogen- and mutagen-induced conversion to anchorage independence (2). These results suggest that multiple steps must occur for immortalized cells to become tumorigenic.

To understand the genetic changes involved in carcinogen-induced neoplastic transformation, we have examined two Syrian hamster cell lines established after carcinogen treatment. We report here that normal, diploid SHE cells have the ability to suppress anchorage independence and tumorigenicity of a highly tumorigenic, benzo[*a*]pyrene-transformed cell line when the two cell types are fused together. Immortal cell lines at early passages also possess this tumor-suppressive function. At later passages before the immortal cells acquire anchorage independence or tumorigenicity, the cells lose the ability to suppress the transformed cells in hybridization

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Abbreviations: P, passage; HAT, hypoxanthine/aminopterin/thymidine; SHE, Syrian hamster embryo.

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experiments. Furthermore, loss of this suppressive function results in an increased susceptibility of the immortal cell lines to transformation following transfection with an oncogene or tumor cell DNA. These results indicate that loss of this suppressive function is a key step in neoplastic progression.

MATERIALS AND METHODS

Cell Culture and Hybridization. SHE cell cultures were established from 13-day gestation fetuses and grown as described (1, 21). The immortalized cells used were DES-4 cells, which were isolated after treatment of SHE cells with diethylstilbestrol (21), and 10W cells, a clone isolated after treatment with asbestos (22, 23). A clone (BP6TM3) of a benzo[*a*]pyrene-induced hamster tumor cell line (1) that is resistant to 1.5 mM ouabain and 10 μg of 6-thioguanine per ml was isolated.

For cell hybrids, 10^6 cells of each parental cell were seeded in 75-cm^2 flasks and fused 24 hr later by treatment for 1 min with 5 ml of 41.7% (wt/wt) polyethylene glycol (PEG 1000, Baker) containing 15% dimethyl sulfoxide followed by treatment for 2 min with an additional 5 ml of 25% PEG without dimethyl sulfoxide at 37°C. The cells were washed extensively, grown for 24 hr in growth medium, and then plated at 10^4 cells per plate into selective medium—i.e., complete medium with 0.1 mM hypoxanthine/0.01 mM aminopterin/16 μM thymidine (HAT) plus 1.5 mM ouabain. Hybrid colonies in cultures with both parental cells formed at a frequency of $\approx 10^{-3}$ per cell, whereas no colonies (frequency, $<10^6$) were observed if only one parental cell type was treated and selected in HAT/ouabain medium.

Hybrid clones after 10–14 days were isolated and grown to a sufficient cell number to analyze. The hybrid cells were tested for anchorage independence in soft agar as described (1). Colonies of >50 cells were scored after 2–3 weeks of incubation. The cells were tested for their tumorigenic potential by subcutaneous injection of 10^4 hybrid cells mixed with early passage, normal SHE cells (10^6) into three sites on nude mice (BALB/c *nu/nu*).

DNAs were isolated and purified as described by Schwab *et al.* (24). DNA transfections were performed by the calcium phosphate precipitate method (17, 25, 26). Cells (1×10^5 per 60-mm dish) were treated with DNA (10–20 μg in 0.5 ml) for 18 hr at 37°C. The medium was removed and the cells were fed with fresh growth medium. After 5 days the cells were assayed for soft agar growth ($1–2 \times 10^6$ total cells assayed) and tumor formation in nude mice (4×10^6 cells per site). Transfection of the cells with pSV2neo plasmid DNA and selection for G418 colonies and cotransfection with the genomic clone (H-1) of Harvey murine sarcoma virus (v-Ha-*ras*) was performed as described (17).

RESULTS

Hybrids Between Normal SHE Cells and Tumorigenic Hamster Cells. For cell hybridization studies we have used normal, diploid SHE cells and a benzo[*a*]pyrene-induced cell line, BP6T, as the tumorigenic parental cell line. The tumor cells were near-diploid and highly tumorigenic with a latency of only 6–8 days (Table 1). We also studied two chemically induced immortal cell lines, DES-4 and 10W. At the passages studied these near-diploid cell lines were nontumorigenic following injection of up to 10^7 cells and failed to grow in agar (frequency of <0.00001 colonies per cell plated).

SHE–BP6T hybrid colonies consisting of >1000 healthy cells were isolated and subcultured. Cells from all of the colonies grew initially but ≈50% (9/20) of the colonies senesced after 1 month, as evidenced by cell enlargement and cessation of growth. These colonies failed to undergo >20 population doublings. In contrast, all hybrid colonies between immortal, nontumorigenic cell lines and BP6T cells were readily subcultured indefinitely. These findings suggest that immortality may be recessive in some of the SHE–BP6T cell hybrids.

Hybrids of SHE–BP6T cells, which did not senesce and could be isolated and grown to a sufficient number of cells for further study, were analyzed for anchorage independence and tumorigenicity. Both phenotypes were suppressed in the hybrids. The hybrid cell grew on plastic with colony-forming efficiencies of 18–46% but grew in agar with greatly reduced colony-forming efficiencies (Table 1). The ability for anchorage-independent growth was reduced by a factor of 10^3 to 10^4 when compared to the ability for anchorage-dependent growth on plastic [i.e., the ratio of anchorage-independent to anchorage-dependent growth was 0.001–0.0001 (Table 1)]. The tumorigenicity of the hybrids was also substantially reduced. Four of five hybrid colonies were nontumorigenic after 30 days (two remained nontumorigenic after 60 days in the nude mice); one formed a tumor on the 25th day, but the tumor later regressed. As a control experiment, BP6T cells were fused with the M3 subclone of BP6T. All of the hybrids grew well in agar and were highly tumorigenic, forming tumors in 6–8 days (Table 1).

The above experiments were performed after the hybrids were grown for ≈20 population doublings. The ability of the hybrids to grow in agar increased with additional growth of the clones. When the clones were assayed at later passages, the increase in anchorage-independent growth was accompanied by increased tumorigenicity (i.e., decreased latency periods). When the average latency periods in nude mice of hybrid cell populations (including BP6T–SHE hybrids, BP6T–BP6T hybrids, and BP6T–DES-4 and BP6T–10W hybrids described below) were plotted against the number of anchorage-independent cells injected per site, the points fit along a straight line derived from a reconstitution experiment with a known number of anchorage-independent BP6T cells injected (Fig. 1). These results indicate that the frequency of anchorage-independent cells is related to the tumorigenicity of the cells, which is consistent with other studies with these cells (27, 28), and further support the use of this *in vitro* phenotype to study the neoplastic transformation of these cells.

Hybrids between immortal, nontumorigenic lines 10W and DES-4 were also studied. All hybrids grew well and colonies were easily isolated. When DES-4–BP6T hybrids were assayed for growth in agar a significant suppression (500–5000 fold) of anchorage-independent growth was observed (Table 1). Likewise, the tumorigenicity or tumor latency of the hybrids was suppressed (Table 1). However, a different pattern was observed in 10W–BP6T hybrids compared to DES-4–BP6T hybrids. All of the 10W–BP6T hybrids grew in agar with a frequency from >1% up to 46% (reduction by a factor of only 2–50). These hybrids were also much more tumorigenic; the latency period was in some cases equal to BP6T parental cells and was increased to 13 days at most.

To confirm the difference between 10W cells and DES-4 cells to suppress anchorage-independent growth of BP6T cells in hybrids, a direct assay of anchorage-independent growth of hybrids was performed. In this assay the cells were fused and after 24 hr, the hybrids were selected in HAT/ouabain medium either on plastic or directly in agar with selective medium. The ratio of hybrids growing in agar (Ag^+) to total hybrids is therefore a measure of the cells' ability to suppress anchorage-independent growth. Control experiments with BP6T–BP6T hybrids resulted in the same number of hybrids in agar and on plastic (ratio = 1.076) (Table 2), whereas the ratio was reduced to 0.0009 for SHE–BP6T hybrids. 10W cells at the passage (P15) examined for the isolated hybrids shown in Table 1 reduced the ratio of Ag^+ hybrids to total hybrids to only 0.711. However, at an earlier

Table 1. Suppression/expression of anchorage-independent and tumorigenic phenotypes in hamster–hamster hybrids

Clone	Plating efficiency, %		Ratio of anchorage-independent to anchorage-dependent growth	Tumorigenicity‡ (average latency period in days)	Modal chromosome number§ (range)
	On plastic *	In agar †			
Parental cells					
SHE	21.2	<0.00005	<0.0001	>30	44 (43–44)
BP6T-M3	70.7	58.0	0.82	7.0	43 (42–46)
DES-4 (P35)	26.6	<0.0001	<0.0001	>30	44 (43–45)
10W (P15)	32.4	<0.0001	<0.0001	>30	45 (44–46)
SHE–BP6T-M3					
Clone 1	29.7	0.015	0.0005	>30	116 (95–124)
Clone 2	42.0	<0.0033	<0.0001	>30	83 (69–85)
Clone 3	44.0	0.057	0.0013	>30	81 (76–84)
Clone 4	46.4	0.01	0.0002	25¶	100 (57–105)
Clone 5	18.7	<0.0033	<0.0002	>30	120 (117–129)
BP6T–BP6T-M3					
Clone 1	55.0	30.7	0.56	7	77 (70–86)
Clone 2	89.3	65.0	0.73	6	78 (77–86)
Clone 3	52.7	22.0	0.42	7	80 (70–84)
Clone 4	58.6	40.7	0.69	8	84 (64–86)
Clone 5	39.0	27.0	0.69	7	121 (83–136)
DES-4 (P35)–BP6T-M3					
Clone 1	39.3	0.176	0.0045	28	86 (69–88)
Clone 2	13.0	0.003	0.0002	>30	82 (70–93)
Clone 3	94.2	0.085	0.0009	26	ND
Clone 4	26.0	0.007	0.0003	>30	83 (77–93)
Clone 5	29.0	0.083	0.0029	27	125 (97–127)
10W (P15)–BP6T-M3					
Clone 1	51.4	17.6	0.342	9.3	87 (78–176)
Clone 2	86.3	1.7	0.02	13.0	110 (106–127)
Clone 3	75.3	2.7	0.035	8.3	ND
Clone 4	72.3	19.7	0.272	6.0	82 (68–84)
Clone 5	96.7	46.0	0.48	7.7	84 (80–89)

ND, not determined.
*10^2 hybrid cells per dish were inoculated in 6-cm dishes, and 7 days later the average plating efficiency was determined from five dishes.
†10^2–10^4 hybrid cells per dish were inoculated into soft agar medium, and 2 weeks later the average plating efficiency was determined from three dishes.
‡10^4 hybrid cells were mixed with 10^6 normal SHE cells and injected subcutaneously into nude mice. The average latency period was determined from three sites when the first nodule was detected.
§Chromosome number was determined from 20 well-spread metaphases.
¶Tumor formed at one site and regressed.

passage (P5), the 10W cells were still effective in suppressing anchorage-independent growth (ratio = 0.0003). Hybrids of 10W (P5)–BP6T cells growing on plastic were isolated and analyzed for anchorage-independent growth and all were suppressed.

DES-4 cells at P35 and P58 were fused with BP6T cells and the ratio of Ag^+ hybrids to total hybrids was 0.0021 and 0.062, respectively. Therefore, at the later passage the ability of the cells to suppress anchorage-independence decreased but not to the degree observed with 10W cells. Subclones of 10W cells (P15) and DES-4 cells (P58) were randomly isolated and tested for the ability to suppress anchorage-independent growth of BP6T cells. As shown in Table 2, subclones of 10W cells and DES-4 cells that varied 60-fold and 20-fold, respectively, in their ability to suppress anchorage independence were identified.

Transfection of BP6T DNA into Different Recipient Cells. To determine if the cells with differing abilities to suppress anchorage-independent growth differed in their susceptibility to transformation by DNA transfection, the cells were transfected with BP6T DNA and assayed for anchorage-independent growth or tumorigenicity. SHE cells treated with BP6T DNA failed to grow in agar or form tumors in nude mice (Table 3). DES-4 cells (P34 and P60) treated with BP6T DNA also failed to grow in agar, but the BP6T DNA-treated cells at P60 formed tumors in nude mice after a latency period of 3 weeks, whereas control treated cultures remained nontumorigenic. 10W cells at P17 treated with BP6T DNA grew in agar and were tumorigenic with a latency of 2 weeks, whereas control treated 10W (P17) cells were negative for both end points. BP6T DNA-treated 10W (P6) cells also were negative. The DNA from agar-positive clones of 10W cells treated with BP6T DNA also induced anchorage-independent growth of 10W cells in a second-round transfection, whereas DNA from 10W or SHE cells was negative.

When transfected with v-Ha-*ras* DNA, 10W cells, which are not effective in suppressing tumorigenicity and anchorage-independent growth (sup^-), grew in soft agar with a 1000-fold higher frequency than DES-4 (sup^+) cells (Table 4). This difference was unrelated to the efficiency of uptake and expression of DNA since the frequency of G418-resistant colonies following transfection with pSV2neo was greater for DES-4 cells (Table 4).

DISCUSSION

Based upon the results of this communication we propose the hypothesis that chemically induced neoplastic transformation of SHE cells involves at least three steps: (*i*) induction of immortality, (*ii*) activation of a dominantly acting, trans-

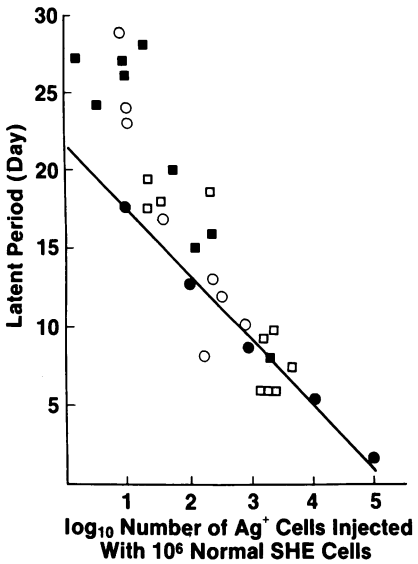

FIG. 1. Relationship for Syrian hamster cells between frequency of anchorage-independent growth and latency period for tumor formation in nude mice. Known numbers of anchorage-independent BP6T cells were mixed with 10^6 normal, nontumorigenic SHE cells and injected subcutaneously into BALB/c *nu/nu* mice. The average latency period for tumor formation was determined and plotted (●), and a straight line plot was obtained. The latency period and frequency of colonies in agar was obtained from different hybrid clones [BP6T–SHE (○), BP6T–10W (□), and BP6T–DES-4 (■)] at different passages and plotted on the same graph. For the most part, the data with hybrids show a good correlation with the predicted curve, with the possible exception of a longer latency period than predicted for populations exhibiting a low frequency of growth in agar. These results indicate that anchorage-independent growth is a good indicator of tumorigenic potential of Syrian hamster cell lines.

forming oncogene, and (*iii*) loss of a tumor suppression function.

The first two changes, immortality and transformation, are consistent with the model of Land *et al.* (4) and Ruley (5) and consistent with our findings that *ras* plus *myc* in combination, but not alone, can induce neoplastic transformation of SHE cells (15). Furthermore, carcinogen-induced immortality of these cells has been shown to be an important step in the

Table 2. Suppression/expression of anchorage-independent phenotype in hybrids of BP6T-M3 cells and various other cells

Cell line fused to BP6T-M3 cells	Hybrid frequency* ($\times 10^{-4}$)	Ag^+ hybrid frequency† ($\times 10^{-4}$)	Ratio of Ag^+ frequency to total hybrid frequency
BP6T	35.2	37.9	1.076
SHE (P5)	7.9	0.007	0.0009
10W (P5)	17.6	0.005	0.0003
10W (P15)	9.9	7.08	0.715
DES-4 (P35)	25.6	0.054	0.0021
DES-4 (P58)	22.4	1.39	0.062
10W clone 5	14.0	0.15	0.01
10W clone 4	28.7	19.2	0.67
DES-4 clone 4	4.3	0.05	0.012
DES-4 clone 5	4.0	1.05	0.26

Each value represents the mean number from three separate experiments.

*Hybrid frequency was determined from the number on colonies growing on plastic dishes in HAT/ouabain medium at 2 weeks after selection.

†Ag^+ hybrid frequency was determined from the number of colonies growing in agar containing selective (HAT/ouabain) medium at 3 weeks after selection.

Table 3. Susceptibility of different hamster cells to transfection by DNA

DNA source	Recipient cells	Average frequency of Ag^+ colonies* per 10^7 cells: DNA treated	Average frequency of Ag^+ colonies* per 10^7 cells: Control treated
BP6T-M3	SHE (P3)	<3.3	<3.3
	DES-4 (P34)	<1.7	<1.7
	DES-4 (P60)	<1.2	<1.2
	10W (P6)	<5	<5
	10W (P17)	54	<1
10W Ag^+ (BP6T)†	10W (P17)	35	<5
10W (P17)	10W (P17)	<3.3	<3.3
SHE (P3)	10W (P17)	<2.5	<3.3

Five days after transfection by the calcium phosphate method, cells were replated into 0.3% agar medium (2×10^5 cells per plate). Each value represents mean number obtained from two or three separate experiments. Control treatments were with calcium phosphate precipitate alone.

*Frequency per 10^7 cells; when no colonies were detected the frequency was expressed as <1 per number of cells assayed.

†Secondary transfection with DNA from agar-positive clone isolated from 10W (P17) cells following transfection with BP6T DNA.

neoplastic progression of these and other cells (1, 6, 17, 29, 30). However, several lines of evidence indicate that an additional change is required for neoplastic transformation. Our three-step model described above is supported by the following observations. (*i*) Cells neoplastically transformed by v-Ha-*ras* plus v-*myc* have a nonrandom loss of chromosome 15 (15). (*ii*) Immortal cell lines are neoplastically transformed by v-Ha-*ras* oncogene alone, but the susceptibilities of cell lines vary (17). (*iii*) Tumorigenicity and anchorage-independent growth are recessive traits in hybrids between tumorigenic cells and normal SHE cells. (*iv*) Some, but not all, immortal cells can suppress tumorigenicity. This ability decreases with passaging of immortal cell lines and subclones are heterogeneous in their ability to suppress transformation. (*v*) Susceptibility of immortal cell lines to neoplastic transformation by DNA transfection with viral oncogenes or tumor DNA is inversely correlated with suppressive ability to cell–cell hybrids.

For a number of years, the recessive nature of tumorigenicity has been indicated by cell–cell hybridization experiments (8–14). Yet, the discovery of transforming genes (oncogenes) is predicated on the dominantly acting nature of these genes in DNA transfection experiments (3). Several possible explanations (which are not mutually exclusive) may exist for these seemingly disparate findings. (*i*) The ability to suppress the tumorigenic phenotype may depend on the genes activated. For example, *ras*-transformed cells are suppressed in hybrids with normal cells (13), whereas cells transformed by DNA viruses are sometimes not (31, 32). (*ii*) The expression of the neoplastic phenotype may depend on the dosage of the putative suppressor and transforming genes. Results of certain studies are consistent with this hypothesis (33, 34). (*iii*) The putative suppressor gene is lost either during the transfection process or during the selection for the transformed cells. The nonrandom loss of chromosome 15 in *ras*-plus-*myc*-induced Syrian hamster tumor cells may be an example of this mechanism (15). (*iv*) The ability of a cell to suppress the tumorigenic phenotype may be dependent on the stage of progression of the cell. The results of the present communication illustrate this possibility.

The implication of the latter explanation is significant in the design of new cellular systems for detection of oncogenes by DNA transfection. The NIH 3T3 assay detects oncogenes from <20% of all human tumors (35). One possible explana-

Table 4. Susceptibility of immortal cells to transfection by different DNAs

Recipient cells	Phenotype*	G418-resistant colonies† with pSV2neo	Agar colonies‡	
			v-Ha-*ras*	BP6T DNA
DES-4 (P55)	sup^+	3×10^{-3}	2.5×10^{-6}	$<1 \times 10^{-6}$
10 W (P15)	sup^-	9×10^{-4}	3.8×10^{-3}	54×10^{-6}

*Cells either suppress (sup^+) or fail to suppress (sup^-) tumorigenicity and anchorage-independent growth of BP6T cells following cell hybridization.
†Cells were transfected with pSV2neo as described (17). Data are expressed as colonies per μg of DNA transfected.
‡Cells were transfected with v-Ha-*ras* (17) or BP6T DNA (as described in Table 3) and selected 3–5 days later for growth in agar.

tion for this finding is that part of the competence of the NIH 3T3 for detecting oncogenes results from the inability of these cells to suppress certain oncogenes, such as *ras*, or to the facility with which this ability is lost during transfection assays. These cells may suppress other oncogenes more efficiently and therefore these are not detected in this assay.

Our results have shown that the tumor suppression function is retained after the cells have escaped senescence and are immortal, indicating that tumor suppression and senescence are separable phenotypes. However, ≈50% of the hybrids between normal SHE cells and BP6T cells senesced, which suggests that immortality is a recessive trait in these cells. Similar results with human cells have been reported (36). The tumorigenicity of the hybrids that senesce could not be determined but it is assumed that these cells are nontumorigenic. The factors or genes responsible for senescence may represent a separate mechanism for tumor suppression than the ones operative in the hybrids that did not senesce.

The most significant finding of the present communication is that the loss of a tumor-suppressive function is a key step in the neoplastic progression of these cells. As immortal cells are passaged in culture, selection of cells that have lost this function occurs. The basis of this loss and the reasons for the selective advantage of these cells are important questions for future study. Further studies on the nature and mechanism of this suppressive function and isolation of the genes responsible for this function are also needed.

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