

# Relationship of Contact Inhibition to Tumor Transplantability, Morphology, and Growth Rate<sup>1</sup>

Robert E. Pollack and George W. Teebor

Department of Pathology, New York University Medical Center, New York, New York 10016

## SUMMARY

Sublines of BHK21 hamster cells were derived by treatment with 5-fluoro-2'-deoxyuridine. These sublines demonstrated increased contact inhibition *in vitro*. The tumors resulting from injection of these sublines were examined, and cells from the tumors were returned to culture. Sublines with increased contact inhibition were less efficient at initiating tumors.

All tumors, however, had an identical morphology and pattern of growth. Although contact inhibition *in vitro* was inversely correlated with capacity for initiation of *in vivo* growth, it did not affect morphology or pattern of tumor growth.

After growth as solid tumors, cells returned to culture were found to retain their original degree of contact inhibition, indicating that passage through the animal had not led to selection of a common transplantable cell type.

## INTRODUCTION

Contact inhibition of cell division in culture (9) has been correlated with capacity for growth *in vivo* as a solid tumor. Cell lines which grow to a high saturation density in culture have a high efficiency of transplantability while highly contact-inhibited lines do not grow as solid tumors (1-3, 7, 8, 10, 11).

In a previous experiment contact-inhibited sublines derived from a densely growing hamster tumor line were much less transplantable than the parent line (7). The sublines were derived by treating the transplantable line with 5-fluoro-2'-deoxyuridine (FUdR), a competitive inhibitor of thymidylate synthetase. This procedure killed cells synthesizing DNA. The progeny of those cells not synthesizing DNA during FUdR incubation included sublines with a high degree of contact inhibition. Such variant sublines retained a high degree of contact inhibition in culture and grew far less effectively as solid tumors *in vivo* (7).

However, some tumors did appear upon injection of contact-inhibited variant cells. These tumors afforded us the opportunity to ask whether such tumors differed in morphology

from those of the parent line and whether they showed less tendency for local invasiveness. With these cell lines, it was possible to ask whether any features of tumor growth other than the capacity for initiation of growth (4, 5) were correlated with contact inhibition and whether the degree of contact inhibition of the injected cell lines was maintained on return to culture.

## MATERIALS AND METHODS

Cell cultures were maintained on Dulbecco and Vogt's modification of Eagle's medium supplemented with 10% calf serum in 20-sq cm plastic Petri dishes at 36.5°C. The medium was changed twice weekly. BHK21, the parent of the lines studied here, is a spontaneous, established cell line of hamster kidney origin (11). The tumorigenic subline of BHK21 used in these experiments, line B, was transplantable at 10<sup>2</sup> cells/animal and had a high saturation density in culture, 60 × 10<sup>4</sup> cells/sq cm.

**Selection of Contact-inhibited Cell Lines.** Sublines of high contact inhibition were selected from cell line B by the FUdR procedure (7). A superconfluent plate of B was incubated with 25 μg/ml FUdR and 250 μg/ml uridine in regular medium for two days. The FUdR concentration was high enough to kill dividing cells; nevertheless, many cells survived the drug and upon transfer gave rise to colonies. Not all surviving colonies displayed parental morphology.

One variant clone, Fl<sup>1</sup>B1, was isolated for its property of reduced maximal cell density. In mass culture Fl<sup>1</sup>B1 had a lower saturation density than B (Table 1) but contained a minority of cells which still grew into dense parental colonies. Two contact-inhibited colonies were therefore recloned from Fl<sup>1</sup>B1. These, Fl<sup>1</sup>B11 and Fl<sup>1</sup>B12C, both had a stable, low saturation density of 5 × 10<sup>4</sup> cells/sq cm (Table 1).

**Saturation Density.** Hemocytometer counts of cell density were made on trypsinized sister cultures. Saturation densities were averaged from cultures in a one-week period during which cell density did not increase.

**Tumor Production.** Growing cultures of cell lines were injected into the right thigh of 20-day-old randomly bred female Syrian hamsters, at 10<sup>6</sup>, 10<sup>4</sup>, and 10<sup>2</sup> cells per animal. All injections were in 0.2 ml of medium plus 0.5% fetal calf serum. The capacity for growth *in vivo* was determined as a function of the time required for cells to produce tumors of 1 cm in diameter in one-half of the animals injected (half-positive time) and the fraction of animals which ultimately

<sup>1</sup>This study was supported by Research Grants 5T0-5-GM 01 668-05, 2F2-A1-33,222 (R. P.), and CA-10978-01 (G. T.) from the USPHS.

Received February 3, 1969; accepted June 6, 1969.

Table 1

Cells	Before <i>in vivo</i> passage		After <i>in vivo</i> passage	
	Saturation density (cells/sq cm X 10 <sup>4</sup> )	Doubling time (days)	Saturation density (cells/sq cm X 10 <sup>4</sup> )	Doubling time (days)
B	50	2	25	1.6
Fl <sup>1</sup> B1	20	2	ND	ND
Fl <sup>1</sup> B11	5	3	4	3
Fl <sup>1</sup> B12C	5	1.5	4	1.4

Saturation densities and doubling times of cell lines of BHK hamster fibroblast cells before and after one *in vivo* passage. ND, not done.

bore tumors (Table 2). Palpable tumors developed at the site of injection 4 to 14 weeks later. Animals were observed for 30 weeks after injection. Animals were anesthetized with ether, and tumors were excised for pathologic examination at 4, 7, and 10 weeks after injection.

**Recovery of Cells from Tumors.** Animals injected with 10<sup>4</sup> cells were sacrificed in the 10th week. Tumors were excised, trypsinized, and placed in culture (12). After a short lag period cultures from tumors originating from B, Fl<sup>1</sup>B11, and Fl<sup>1</sup>B12C developed well. Saturation densities were determined by a hemocytometer count of trypsinized cultures.

Table 2

Cell Line	Number of cells injected	Time for half of animals given injection to develop 1-cm tumors (weeks)	Number of animals bearing tumors at 28 weeks
B	10 <sup>6</sup>	4	6/6
	10 <sup>4</sup>	4.5	6/6
	10 <sup>2</sup>	8	4/6
Fl <sup>1</sup> B1	10 <sup>6</sup>	5	6/6
	10 <sup>4</sup>	4.5	6/6
	10 <sup>2</sup>	10	6/6
Fl <sup>1</sup> B11	10 <sup>6</sup>	5	5/6 <sup>a</sup>
	10 <sup>4</sup>	10	6/6
	10 <sup>2</sup>		1/6
Fl <sup>1</sup> B12C	10 <sup>6</sup>	4.5	6/6
	10 <sup>4</sup>	6.5	6/6
	10 <sup>2</sup>		1/6

Growth of cells in weanling hamsters.

<sup>a</sup>One animal received 10<sup>6</sup> cells of Fl<sup>1</sup>B11 and was sacrificed at the 7th week. At that time no evidence of tumor was seen.

## RESULTS

**Tumor Production.** Fl<sup>1</sup>B11 and Fl<sup>1</sup>B12C sublines were more contact inhibited than Fl<sup>1</sup>B1, which in turn was more contact inhibited than the transplantable parent line B (Table 1). When 10<sup>6</sup> cells were injected, all animals eventually developed tumors, and the half-positive times of the four lines were

identical. When 10<sup>4</sup> cells were injected, the half-positive times of Fl<sup>1</sup>B11 and Fl<sup>1</sup>B12C were about twice as long as the half-positive time of B, but all lines still grew into tumors in all animals injected. At injections of 10<sup>2</sup> cells, Fl<sup>1</sup>B11 and Fl<sup>1</sup>B12C did not yield half-positive times since only one of the six animals injected developed a tumor in each case. The half-positive times of the B and Fl<sup>1</sup>B11 lines (8 and 10 weeks) injected with 10<sup>2</sup> cells were comparable to the half-positive times of the Fl<sup>1</sup>B11 or Fl<sup>1</sup>B12C cells injected with 10<sup>4</sup> cells.

More Fl<sup>1</sup>B11 or Fl<sup>1</sup>B12C cells than B or Fl<sup>1</sup>B1 cells were needed to guarantee that the majority of animals injected would eventually develop tumors. These data show that Fl<sup>1</sup>B11 and Fl<sup>1</sup>B12C are less tumorigenic than the parent line B or the mixed line Fl<sup>1</sup>B1.

Tumors growing in animals injected with contact-inhibited sublines grew as rapidly as tumors from B or Fl<sup>1</sup>B1. Once detected, all tumors grew progressively until the time of sacrifice.

**Pathology.** Tumor-bearing animals were sacrificed, and their tumors excised, at 4, 7, and 10 weeks postinjection. All tumors were poorly encapsulated and multilobular and contained multiple areas of necrosis. Histologic examination revealed a moderately pleomorphic sarcoma with some giant cell forms growing in a loose fascicular pattern. Tumors from the four lines were indistinguishable, and all were similar to the BHK21 sarcoma (3, 10).

**Properties in Culture of Cells Recovered from Tumors.** Because the tumors were histologically indistinguishable, it was thought at first that they had all grown out by selection of a few cells of the B type present at injection in Fl<sup>1</sup>B11 and Fl<sup>1</sup>B12C. When returned to culture, however, cells of tumors gave rise to lines as contact inhibited as the lines originally injected (Table 1). Cells from Fl<sup>1</sup>B11 and Fl<sup>1</sup>B12C tumors retained the low saturation density of their parent cell lines, while cells from B tumors grew to an eight-fold higher density (Table 1). The doubling times for recovered lines were not different from those of the injected lines (Table 1).

## DISCUSSION

Contact-inhibited sublines of BHK were less able than the densely growing parent line to initiate growth as tumors. The tumors resulting from injection of a larger number of cells of the contact-inhibited sublines did not manifest differing degrees of invasion, or a morphology differing from tumors resulting from injection of the parent line. The rate of growth

of all tumors was identical once the tumors reached a diameter of 1 cm. This indicates that the capacity to initiate growth as a tumor *in vivo* is a property of cultured cells that is distinct from other properties of malignant tumor growth (4, 5), such as tumor morphology and *in vivo* growth rate.

The degree of contact inhibition of injected cell lines was not altered in one *in vivo* passage as a solid tumor. Any variants with less contact inhibition that might have arisen during tumor growth (4, 6, 8-11) apparently did not have any selective growth advantage thereafter, for they did not become the predominant cell type within the tumors. Studies on the fate of contact-inhibited cells which do not grow upon injection are in progress.

#### ACKNOWLEDGMENTS

The authors are indebted to Dr. Howard Green and Dr. Marvin Kuschner for advice and encouragement.

#### REFERENCES

1. Aaronson, S., and Todaro, G. Basis for the Acquisition of Malignant Potential by Mouse Cells Cultivated *in Vitro*. *Science*, *162*: 1024-1026, 1968.
2. Black, P., and Rowe, W. Increase of Malignant Potential of BHK21 cells by SV40 DNA Without Persistent New Antigen. *Proc. Natl. Acad. Sci. U.S.*, *54*: 1126-1133, 1965.
3. Defendi, U., Lehman, J., and Kraemer, P. "Morphologically Normal" Hamster Cells with Malignant Properties. *Virology*, *19*: 592-598, 1964.
4. Diamandopoulos, G. Comparison of the *in Vitro* Cytology of Hamster Embryo Cell Lines Transformed "Spontaneously" or by SV40. *Am. J. Pathol.* *52*: 663-647, 1968.
5. Diamandopoulos, G., and Enders, J. Studies on Transformation of Syrian Hamster cells by Simian Virus 40 (SV40): Acquisition of Oncogenicity by Virus Exposed Cells Apparently Unassociated with the Viral Genome. *Proc. Natl. Acad. Sci. U. S.*, *54*: 1092-1099, 1965.
6. Macpherson, I. Malignant Transformation and Reversion in Virus Infected Cells. In: Werner H. Kirsten (ed.), *Recent Results in Cancer Research, Malignant Transformation by Viruses*, Volume 6, pp. 1-8. New York: Springer-Verlag, Inc., 1966.
7. Pollack, R., Green, H., and Todaro, G. Growth Control in Cultured Cells: Selection of Sublines with Increased Sensitivity to Contact Inhibition and Decreased Tumor-Producing Activity. *Proc. Natl. Acad. Sci. U. S.*, *60*: 126-133, 1968.
8. Sanford, K., Likely, G., and Earle, W. The Development of Variations in Transplantability and Morphology Within a Clone of Mouse Fibroblasts Transformed to Sarcoma-Producing Cells *in Vitro*. *J. Natl. Cancer Inst.*, *15*: 215-237, 1954.
9. Stoker, M. Short-Range Factors Affecting Cell Growth and Movement. *UICC Monograph*, *6*: 193-203, 1967.
10. Stoker, M., and Abel, P. Conditions Affecting Transformation by Polyoma Virus. *Cold Spring Harbor Symp. Quant. Biol.*, *27*: 375-386, 1962.
11. Stoker, M., and Macpherson, I. The Syrian Hamster Fibroblast Cell Line BHK21 and its Derivatives. *Nature*, *203*: 1355-1357, 1964.
12. Todaro, G., Nilausen, K., and Green, H. Growth Properties of Polyoma Virus-Induced Hamster Tumor Cells. *Cancer Res.* *23*: 825-832, 1963.