Relationship of Contact Inhibition to Tumor Transplantability, Morphology, and Growth Rate

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 vitro and grew 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 $10^2$ cells/animal and had a high saturation density in culture, $60 \times 10^4$ 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, F1 B1, was isolated for its property of reduced maximal cell density. In mass culture F1 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 F1 B1. These, F1 B11 and F1 B12C, both had a stable, low saturation density of $5 \times 10^4$ 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 $10^6$, $10^4$, and $10^2$ 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...
Contact Inhibition and Tumor Characteristics

Table 1

<table>
<thead>
<tr>
<th>Cells</th>
<th>Before in vivo passage</th>
<th>After in vivo passage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saturation density (cells/sq cm ( \times 10^8 ))</td>
<td>Doubling time (days)</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>FI(^1)B1</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>FI(^1)B11</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>FI(^1)B12C</td>
<td>5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

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 \( 10^6 \) 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, FI\(^1\)B11, and FI\(^1\)B12C developed well. Saturation densities were determined by a hemocytometer count of trypsinized cultures.

Table 2

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Number of cells injected</th>
<th>Time for half of animals given injection to develop 1-cm tumors (weeks)</th>
<th>Number of animals bearing tumors at 28 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>( 10^6 )</td>
<td>4</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>( 10^5 )</td>
<td>4.5</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>( 10^4 )</td>
<td>8</td>
<td>4/6</td>
</tr>
<tr>
<td>FI(^1)B1</td>
<td>( 10^6 )</td>
<td>5</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>( 10^5 )</td>
<td>4.5</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>( 10^4 )</td>
<td>10</td>
<td>6/6</td>
</tr>
<tr>
<td>FI(^1)B11</td>
<td>( 10^6 )</td>
<td>5</td>
<td>5/6</td>
</tr>
<tr>
<td></td>
<td>( 10^5 )</td>
<td>10</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>( 10^4 )</td>
<td>10</td>
<td>1/6</td>
</tr>
<tr>
<td>FI(^1)B12C</td>
<td>( 10^6 )</td>
<td>4.5</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>( 10^5 )</td>
<td>6.5</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>( 10^4 )</td>
<td>10</td>
<td>1/6</td>
</tr>
</tbody>
</table>

Growth of cells in weanling hamsters.

\(^a\)One animal received \( 10^6 \) cells of FI\(^1\)B11 and was sacrificed at the 7th week. At that time, no evidence of tumor was seen.

RESULTS

Tumor Production. FI\(^1\)B11 and FI\(^1\)B12C sublines were more contact inhibited than FI\(^1\)B1, which in turn was more contact inhibited than the transplantable parent line B (Table 1). When \( 10^6 \) cells were injected, all animals eventually developed tumors, and the half-positive times of the four lines were identical. When \( 10^6 \) cells were injected, the half-positive times of FI\(^1\)B11 and FI\(^1\)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 \( 10^4 \) cells, FI\(^1\)B11 and FI\(^1\)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 FI\(^1\)B11 lines (8 and 10 weeks) injected with \( 10^2 \) cells were comparable to the half-positive times of the FI\(^1\)B11 or FI\(^1\)B12C cells injected with \( 10^4 \) cells.

More FI\(^1\)B1 or FI\(^1\)B12C cells than B or FI\(^1\)B1 cells were needed to guarantee that the majority of animals injected would eventually develop tumors. These data show that FI\(^1\)B11 and FI\(^1\)B12C are less tumorigenic than the parent line B or the mixed line FI\(^1\)B1.

Tumors growing in animals injected with contact-inhibited sublines grew as rapidly as tumors from B or FI\(^1\)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 FI\(^1\)B11 and FI\(^1\)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 FI\(^1\)B11 and FI\(^1\)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.

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REFERENCES

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