The development of an ascites tumor from a transplantable squamous cell carcinoma of mouse was reported several years ago (9). It appeared then to be the only reported instance of an experimental adaptation of a squamous cell carcinoma to an ascitic form of growth. Because this ascites tumor consisted of a highly anaplastic cellular population, which differed markedly from definitely squamous components of the parent solid tumor, more work was required to throw light on the factors responsible for the observed inconsistencies. Since then several investigators have studied a related ascites tumor, MT890, which was developed subsequently (6, 12—14). Later attempts to produce new ascites tumors from the original transplantable solid carcinoma, MT70, by the direct i.p. inoculations of mice, resulted in the development of new ascitic tumors which were subjected to investigation (11, 15). The present study was undertaken before these new tumors were developed. Therefore, new methods were sought for the adaptation of cells from a transplantable squamous cell carcinoma of mouse to growth in the intraperitoneal environment. It soon became apparent that a tissue culture cell line already developed from this tumor (3) provided a logical source of inoculum for testing its ability to develop ascitic tumors. This paper is concerned with the technics used to obtain ascites tumors from tissue culture cell lines and describes the development of new tissue culture cell lines derived in the course of this work.

Other reports mention the ability of cells from certain ascites tumors to grow in tissue culture (1, 2, 7, 8). However, no reports of a successful use of tissue culture cell lines for the production and study of ascites tumors originating from a squamous cell carcinoma were found.

The Usefulness of Tissue Culture Cell Lines in the Development of Ascites Tumors from a Transplantable Squamous Cell Mouse Carcinoma

M. R. A. FERNANDES AND IRENA KOPROWSKA

(Department of Pathology—Cytology Section, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania)

SUMMARY

Ascites tumors were successfully produced by using tissue culture Cell Line 70, obtained from a transplantable squamous cell carcinoma of the mouse. The derived tissue culture cell lines with described tumorigenic properties were also developed from ascitic and solid tumors resulting from the inoculation of mice with Cell Line 70. The use of cell lines for the production of ascites tumors from squamous cell carcinoma is valuable for the comparative study of tumor differentiation under in vivo and in vitro conditions.

MATERIAL AND METHODS

Cultures of Cell Line 70 were used. The development of this Cell Line from transplantable mouse tumor MT70 and its characteristics were reported previously (3).

Calf serum was inactivated for 4 hr at 56°C and preserved at 4°C. A 25% sterile solution of 1:250 trypsin in Gey’s balanced salt solution without calcium and magnesium, was preserved frozen at -20°C; it was adjusted to pH 7.3. Eagle’s minimum essential medium with 10% calf serum added, was used as a nutrient for derived cell lines.

All media were supplemented with 100 units of penicillin and 100 mg of streptomycin/ml.

Female virgin C3H/He mice, obtained from the Jackson Memorial Laboratory when 4-6 weeks old, were caged in groups up to 10, and given Purina laboratory chow and tap water ad libitum. They were kept in air conditioned quarters.

The cells were cultured in milk dilution bottles. Trypsin solution was used to detach the cells from the glass, and cells were counted in a hemacytometer according to standard technics. Cells grown on coverslips in Leighton tubes were used for the study of stained preparations.

As previously reported, C3H mice received cells from Cell Line 70 s.c.; this was followed by the growth of tumors which were so poorly differentiated histologically that their classification as carcinomatous rather than sarcomatous tumors was arbitrary. It was based more upon their known origin than upon morphologic or histochemical considerations (3). Pieces of these tumors were cut into minute fragments with scissors. These fragments were suspended in the described medium. The cells were further separated by vigorous pipetting. The resulting suspension was distributed into milk dilution bottles for the development of new lines derived from Cell Line 70.

1 This study was supported by a research grant, CY-3654, from the National Cancer Institute, NIH, Bethesda, Md.

Received for publication October 19, 1964.
In order to develop ascites tumors from Cell Line 70, and to produce derived tissue culture cell lines from the obtained ascites tumors, the following technic was used. A counted cellular inoculum prepared from Cell Line 70 was used for i.p. injections to produce ascites tumors. The number of cells per inoculum used for injecting animals varied according to the experiment. The volume of the inocula was constant for a given route, i.e., 0.2 ml i.p. and 0.1 ml s.c. The successfully produced tumors were maintained by serial passage (i.p.) from mouse to mouse. Tuberculin syringes with 20-gauge needles were used to withdraw fluid from the abdominal cavity in order to develop tissue culture cell lines derived from these new ascites tumors. The medium was added to this fluid until a standardized concentration of 200,000 cells/ml was obtained. This suspension was distributed into milk dilution bottles and into Leighton tubes.

The chromosomes were studied by Moorhead's method (10). Bottles containing cells in active division (usually 72 hr after the subculture was made) were used for counting chromosomes.

RESULTS

The inoculation of mice with different concentrations of cells resulted in the development of 2 types of tumors, depending upon route of inoculation.

Subcutaneous route.—Of 50 mice receiving from 100 to 30,000 cells, 46 developed solid subcutaneous tumor nodules within 12-43 days after inoculation. The tumors usually developed early when the number of inoculated cells was large. Of the 4 mice which did not develop tumors during the 180-day observation period, 2 received 100- and 2 1000-cell inocula. The subcutaneous tumors produced by Cell Line 70 grew well in tissue culture. Two lines were derived from subcutaneous tumors produced by the 4th and 5th subcultures of Cell Line 70. These were designated 7OTCSC1 and 7OTCSC11. The 2nd one is still being maintained after 106 subcultures for 26 months. The 1st was stored frozen after 56 subcultures. Fig. 1 shows the histologic appearance of a tumor nodule from which 7OTCSC11 was derived. The multiplication index of these derived cell lines initially was more rapid than in the case of Cell Line 70. Later all 3 lines had the same rate of multiplication.

These "subcutaneously" derived cell lines have a similar morphology, maintain a similar ability to produce subcutaneous solid tumors, but have a greater ability to produce ascites tumors when reinoculated in mice than does Cell Line 70. The results shown in Tables 1 and 2 may be summarized briefly as follows:

The solid tumor inducing properties were similar. A concentration of cells above 12,500 induced tumors within 2-3 weeks in all of the tested mice, regardless of which cell line was used. The latency period was reduced to 10 days by doubling the concentration of cells. Occasional tumors arose within 10 days, if the concentration of cells was diminished to 100.

The tumors produced by 7OTCSC1 and 7OTCSC11 were histologically very similar to the tumor nodules produced by the s.c. inoculation of cells originated from Cell Line 70. Although their component cells varied in the amount of cytoplasm, and in the prevalence of a mosaic or a bundle type of grouping from one portion of the tumor to another, all these tumors were very similar. They looked more like sarcomas than carcinomas, and differed significantly from their precursor—transplantable squamous cell carcinoma MT70.

The ascites tumor-inducing properties appeared to be enhanced. The derived cell lines produced ascitic fluid more uniformly and with smaller inocula than Cell Line 70. The gross and microscopic appearance of ascites tumors produced by the derived cell lines was generally similar to that of the ascitic tumors produced by Cell Line 70.

The results of chromosome counts were obtained from 150 metaphases. These results were also similar to the counts obtained for Cell Line 70, and were as follows:

In Table 1, the results of chromosome counts obtained for Cell Line 70, and were as follows:

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Number of Cells per Inoculum (0.1 ml)</th>
<th>Number of Animals Inoculated (i.p.)</th>
<th>Number of Animals Developing Tumors</th>
<th>Time of Tumor Appearance (days after inoc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>30,000</td>
<td>3</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>15,000</td>
<td>3</td>
<td>3</td>
<td>11-14</td>
</tr>
<tr>
<td></td>
<td>7,500</td>
<td>3</td>
<td>3</td>
<td>17-41</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>3</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>7OTCSC1</td>
<td>100,000</td>
<td>3</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>50,000</td>
<td>3</td>
<td>3</td>
<td>10-11</td>
</tr>
<tr>
<td></td>
<td>25,000</td>
<td>3</td>
<td>3</td>
<td>10-21</td>
</tr>
<tr>
<td></td>
<td>125,000</td>
<td>3</td>
<td>3</td>
<td>14-26</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>3</td>
<td>1</td>
<td>47</td>
</tr>
<tr>
<td>Control (Nutrient medium)</td>
<td>—</td>
<td>12</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 2 compares the results of ascites tumor-inducing properties of Cell Line 70 and derived cell line 7OTCSC1 in C3H mice.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Number of Cells per Inoculum (0.1 ml)</th>
<th>Number of Animals Inoculated (i.p.)</th>
<th>Number of Animals Developing Tumors</th>
<th>Time of Tumor Appearance (days after inoc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>100,000</td>
<td>3</td>
<td>3</td>
<td>8 (in 2 mice) 59 (in 1 mouse) 8</td>
</tr>
<tr>
<td></td>
<td>50,000</td>
<td>3</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3,500</td>
<td>3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>7OTCSC11</td>
<td>100,000</td>
<td>3</td>
<td>3</td>
<td>23-26</td>
</tr>
<tr>
<td></td>
<td>50,000</td>
<td>3</td>
<td>3</td>
<td>23-31</td>
</tr>
<tr>
<td></td>
<td>25,000</td>
<td>3</td>
<td>3</td>
<td>19-41</td>
</tr>
<tr>
<td></td>
<td>12,500</td>
<td>3</td>
<td>3</td>
<td>23-37</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>3</td>
<td>3</td>
<td>23-54</td>
</tr>
<tr>
<td>Control (Nutrient medium)</td>
<td>—</td>
<td>12</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

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new cell lines differ markedly from the cellular populations 
vided fluids for the reinoculation of other mice in order to 
tures, respectively. The cellular populations of these 
under low magnification through the phase contrast 
cultures. The new cell lines were designated 701P1 and 
initiate new ascites tumors and tissue culture lines. These 
ascites tumors were propagated for many generations by 
i.p. passage. One of them is still being maintained. The 
resulting tissue cultures were successful in both instances. 
Consequently, 2 additional cell lines were derived from the 
i.p. inoculation of Cell Line 70 in 5th and 16th subcul 
tures. The new cell lines were designated 701P1 and 
701P11. They are presently in their 98th and 73rd subcul 
tures, respectively. The cellular populations of these 
new cell lines differ markedly from the cellular populations 
characteristic of Cell Line 70. The differences observed 
under low magnification through the phase contrast 
microscope may be summarized as follows: 
Derived Cell Line 701P1 and subsequently subderived 
Cell Line 701P11 consisted predominately of epithelial 
like cells and relatively few fibroblast-like cells. The 
epithelial-like cells were mononuclear, binucleated, and 
multinucleated. Giant cells were rare in the initial subcul 
tures but became more frequent in later subcultures, 
reaching about 20% of the cellular population. Almost 
all of the giant cells were multinucleated with rosette-like 
nuclear arrangements in their centers. Micronuclei were 
also observed.

In derived Cell Line 70IP11 and in the later subderived 
Cell Line 70IP11, epithelial-like cells were strikingly pre 
dominant, and fibroblast-like cells were rare. Occasional 
giant cells were present.

In the later subcultures a marked degree of pleomor 
phism was observed. Figs. 2–5 illustrate the general ap 
pearance of Cell Line 70, derived Cell Line 70IP11 and 
derived Cell Line 70IP11.

The subderived cell lines grew well. The time re 
quired to make the initial subcultures was shorter than 
the time required to make the same number of subcul 
tures from the parental cell line.

The general impression of a highly pleomorphic cel 
lular population in derived Cell Line 70IP1 and 70IP11 with 
an expected sizeable proportion of polyploid cells was 
well supported by chromosomal counts.

Chromosomes were very numerous and difficult to count; 
their number ranged from 62 to 200.

The reinoculation of mice with these derived cell lines 
(either s.c. or i.p.) resulted in the development of tumors. 
The ascites tumors developed slowly, but their appear 
ance was followed by the prompt death of hosts.

701P1, was produced by cells recovered from an ascites tumor developed though an i.p. inoculation of mice with the cells from the 3rd subculture of derived Cell Line 70IP1.

701P11, was produced analogically to the subderived Cell Line 
70IP1; from the 3rd subculture of derived Cell Line 70IP11.

Of all the ascites tumors obtained through i.p. inocula 
tion of mice with Cell Line 70 and/or derived tissue cul 
ture cell lines, the following were propagated for an ap 
preciable length of time: (a) The ascites tumor correspond 

DISCUSSION

The testing of the tumorigenic properties of cell lines by animal inoculation, and their enhancement in derived 
cell lines, has been reported by other investigators (1, 2, 
7). In the present work, some enhancement of the ability 
to elicit ascites tumors was noted in derived Cell Lines 
70TCS 7 and 70TCS 11. In addition, the development of ascites tumors from the tissue culture cell line described 
in this paper provides a new method for the development of 
ascites tumors from a squamous cell carcinoma. It also 
offers an opportunity to study cellular populations of 
common origin propagated simultaneously within an 
animal host and in tissue culture.

The authors (4) previously observed a cellular trans 
formation in vitro, which lead to the development of a 
tissue culture cell line from a benzpyrene-induced, poten 
tially malignant lesion of a mouse uterine cervix. A 
cellular “transformation” pattern characterized by the 
appearance and multiplication of small, undifferentiated 
cells and their disorderly growth and accumulation, and 
the presence of giant cells and bizarre forms among better 
differentiated epithelial-like cells, was described. It was 
shown to be a faithful replication of otherwise familiar 
in vivo events fully reproducible in experimental animals. 
These events are well known to pathologists and are 
morphologically recognizable as basal and/or reserve cell 
hyperactivity, epithelial dysplasia and/or carcinoma in 
situ (4). It would thus appear that one may reconstruct 
in vitro a link in the chain of events involved in the neo 
plastic progression at the stage of a potentially malignant, 
noninvasive lesion (5). Parallel in vivo and in vitro cellu 
lar studies of ascites tumors may lead to the discovery of 
another link at a more advanced stage of neoplastic 
progression.

ACKNOWLEDGMENTS

The authors are indebted to Mr. Claude Koprowski for the de 
velopment and printing of photomicrographs as well as for his 
assistance in editing this manuscript.

REFERENCES

1. Barski, G., Biedler, J. L., and Cornefert, F. Modification of 
Characteristics of an in Vitro Mouse Cell Line after an In 
2. Cailleau, R., and Costa, F. Long Term in Vitro Cultivation of 
3. Fernandes, M. A. R., and Koprowska, I. Chemically Induced 
Transplantable Murine Carcinoma Cell Line 70. Acta Cytol., 
4. Fernandes, M. A. R., and Koprowska, I. Tissue Culture Stud-

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FIG. 1.—Histologic appearance of an undifferentiated subcutaneous tumor nodule produced by the 5th subculture of Cell Line 70, from which derived Cell Line 70TCSII was developed. H and E. × 125.

FIG. 2.—Cell Line 70, showing general appearance of the 153rd subculture. Jacobson stain. × 125.

FIG. 3.—Derived Cell Line 70IP1, showing general appearance of the 89th subculture. Jacobson stain. Giant multinucleated cell with central nuclei arranged in rosette-like fashion is seen. Micronuclei are also present in giant cell. × 125.

FIG. 4.—The same giant cell as in Fig. 3. × 500.

FIG. 5.—Derived Cell Line 70IP11, showing general appearance of the 55th subculture. Jacobson stain. × 125.

FIG. 6.—Ascites tumor 70IP1, obtained from the subderived Cell Line 70IP1, and still maintained in animal passages. Papanicolaou stain. × 500.

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Cancer Res 1965;25:444-449.

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