Human Cells in Continuous Culture

I. Derivation of Cell Strains from Esophagus, Palate, Liver, and Lung*

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Human and animal cells of various types in continuous culture are useful for both virus and cancer research. Reports (10, 13) of variations in long-cultivated cells suggest that, like inbred mice, these should be repeatedly described by subline and that new strains should be added to the list as often as possible. This report concerns (a) the derivation of established human cell strains and clonally initiated substrains now maintained in this laboratory; (b) some technics for and results of propagation of strains from surgical specimens; and (c) the origin, development, and some characteristics of three human cell strains previously originated from normal tissue.

MATERIALS AND METHODS

Preparation of Hanks’ solution (BSS), maintenance solution (MS), chick embryonic extract (EE) and serum, and the enumeration and dispensing of cells for cultures in tubes or bottles have been reported (16).

Yeast extract basal medium (YEM) contained 10 parts of 1 per cent Difco Yeastolate in distilled water, 2.5 parts 10 per cent glucose in distilled water, and 87.5 parts BSS (12). For additional buffering when needed, 0.113 gm. per cent sodium acetate and 0.064 gm. per cent sodium pyruvate were added to the BSS.

Human or other serum taken from single donors for establishment or maintenance of new cell strains was pretested for toxicity to cells in culture.

Growth medium contained 20 parts of human serum (HuS), 76 parts YEM, and 4 parts 1.4 per cent sodium bicarbonate in distilled water. Human, chicken, monkey, or calf serum, 3–5 parts, was combined with 5 parts 1.4 per cent sodium bicarbonate and 90 parts YEM for prolonged maintenance of cells with minimum multiplication. All media contained 100 units penicillin and 100 μg streptomycin/ml.

Source tissue for primary culture was received as biopsies taken for diagnosis from patients admitted to University of Minnesota Hospitals. Specimens were stored for 1 hour or less in HuS-20, YEM-80 at 4° C. before use.

Primary culture was initiated with explants 0.5–1.5 mm. in diameter, cut from tissue wetted with embryonic extract by crossed scalpel blades handled like scissors. Coverslips in Leighton tubes were coated with a minimum amount of human or chicken plasma; six explants in EE were arranged on each coverslip. Usually six to ten such tube cultures after incubation contained enough multiplying cells for serial passage.

Serial passage was started in duplicate from two primary cultures. Cells were detached from the parent culture with a rubber policeman to start one passage set, and with trypsin to start the other set. Trypsin (Difco 1:250, 0.1–0.2 per cent in MS-100 at pH 7.4–7.6) was occasionally toxic for cells in primary culture; complete loss of cells was avoided by concomitant use of mechanical detachment. After incubation for 5 minutes or less at 37° C., cells in trypsin solution were diluted in cold YEM medium to stop tryptic action and centrifuged at 1000 r.p.m. or less. For serial passage, tubes or flasks were seeded with cells, at least 10⁶/ml, and resuspended in growth medium. Cells maintained in continuous culture for 12 or more months were considered fully established as a stable strain.

Clonally initiated substrains were obtained by triple isolation from single cells. Clones from isolated single cells were established readily by use of embryonic extract to suspend terminal dilutions of trypsinized cells and plating in thin layers of human or chicken plasma on the glass surface of small petri plates 5 cm. in diameter. The petri plates were placed in wide-mouthed glass jars,
flushed with 5 per cent carbon dioxide, and incubated at 37° C. Plating in thin layers of human or chicken plasma was found to speed colonial multiplication of small numbers of isolated cells. Cell colonies also were grown with or without the aid of a feeder layer, as described by Puck, Marcus, and Cieciura (11).

RESULTS

Cell strains established in continuous culture are maintained as described by Syvertson and Scherer (15); such cells and others now are propagated in YEM medium. The original HeLa strain (5) growth behavior and chromosome pattern of these and other cells have been reported (14).

New human cell strains were initiated from primary cultures of small portions of tissue excised from surgical specimens or biopsies taken for diagnostic purposes from thirteen patients admitted to University of Minnesota Hospitals for elective surgery between November, 1955, and October, 1956. Descriptive data are shown in Table 1.

Culture of tissue from seven of the thirteen patients failed to produce growth. These cultures were made from normal or malignant tissue of the

| TABLE 1 |
| RESULTS OF in Vivo CULTURE OF HUMAN TISSUE BIOPSIES, AND DESCRIPTION OF DERIVED CELL STRAINS |

<table>
<thead>
<tr>
<th>PATIENT NO. AND SEX</th>
<th>DIAGNOSIS</th>
<th>TISSUE</th>
<th>SOURCE</th>
<th>CHARACTERS OF CULTIVATED CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PRINCIPAL CELL TYPE</td>
</tr>
<tr>
<td>1 female</td>
<td>Carcinoma of colon</td>
<td>Ovary</td>
<td>Epithelial</td>
<td>5 days</td>
</tr>
<tr>
<td>2 male</td>
<td>Normal</td>
<td>Lung</td>
<td>Fibrocytic</td>
<td>96 days</td>
</tr>
<tr>
<td>3 male</td>
<td>Cleft palate</td>
<td>Palate</td>
<td>Fibrocytic</td>
<td>19+months</td>
</tr>
<tr>
<td>4 female</td>
<td>Carcinoma of colon</td>
<td>Normal bowel</td>
<td>Fibrocytic</td>
<td>190 days</td>
</tr>
<tr>
<td>5 female</td>
<td>Tracheo-esophageal fistula</td>
<td>Esophagus</td>
<td>Fibrocytic</td>
<td>18+ months</td>
</tr>
<tr>
<td>6 female</td>
<td>Not known</td>
<td>Liver</td>
<td>Epithelial</td>
<td>18+</td>
</tr>
<tr>
<td>16-month infant</td>
<td>Sarcoma of femur</td>
<td>Sarcoma</td>
<td>No culture</td>
<td>8,11</td>
</tr>
<tr>
<td>7 female</td>
<td>Normal</td>
<td>Bowel</td>
<td>No culture</td>
<td>0</td>
</tr>
<tr>
<td>8 female</td>
<td>Plasma-cell hepatitis</td>
<td>Liver</td>
<td>Fibrocytic</td>
<td>6</td>
</tr>
<tr>
<td>9 female</td>
<td>Rectal carcinoma</td>
<td>Carcinoma</td>
<td>No culture</td>
<td>0</td>
</tr>
<tr>
<td>10 female</td>
<td>Normal</td>
<td>Lung</td>
<td>Epithelial</td>
<td>3</td>
</tr>
<tr>
<td>11 female</td>
<td>Bladder papilloma</td>
<td>Bladder</td>
<td>No culture</td>
<td>0</td>
</tr>
<tr>
<td>12 female</td>
<td>Polycystic kidney</td>
<td>Kidney</td>
<td>No culture</td>
<td>5</td>
</tr>
<tr>
<td>13 female</td>
<td>Polycystic kidney</td>
<td>Kidney</td>
<td>No culture</td>
<td>5</td>
</tr>
</tbody>
</table>

of uterine cervical carcinoma supplied by Dr. G. O. Gey in plasma-clot culture has given rise to six parental (Fig. 1) and numerous clonal sub-strains (Fig. 2). Several clonal sublines (Fig. 3) have been derived during 30 or more passages of two parental strains (Fig. 4) of Maben (4) pulmonary adenocarcinoma received from Dr. A. W. Frisch in December, 1955. Chang's conjunctival cell (1) was received in March, 1955, from Dr. John Snyder. These lines have since passed more than 150 generations in this laboratory and many clones have been established from them (Fig. 5). Chang's liver cell (1), received in March, 1955, from Dr. Smadel, has also been used for production of clones many times (Fig. 6) since December, 1955. The liver lines are separated from the donor culture by some 90 transfers. Some features of the gastrointestinal tract, a papilloma of the bladder, and a fibrosarcoma.

Four cell strains were or have been maintained in continuous culture for 7 or less months. One strain from normal lung parenchyma (No. 2) was lost after four transfers over a 3-month period; a second strain from lung tissue (No. 11) has continued through eight passages during the same period (Fig. 7). A human kidney (No. 13) epithelial cell has been maintained for 5 months as a strain of comparatively low reproductive capacity. A fibrocyte strain derived from a piece of esophagus removed from a 1-day-old infant (No. 5) with a tracheo-esophageal fistula grew vigorously for more than 6 months (Fig. 8); this strain was lost without apparent cause after 21 passages.

A strain of esophageal epithelium (Minn. EE)
also was originated from the infant with tracheoesophageal fistula. This cell, now extensively used together with clonal derivatives (Figs. 9, 10) for virus studies in this laboratory, has propagated vigorously through 36 passages. A fibrocyte line (palate fibroblast) derived from a child (No. 3) with cleft palate has been transferred 35 times (Fig. 11) during 19 months of continuous culture, so it may be considered stable. The palate fibroblast grows less rapidly than, and is about a third the size of, the unfortunately lost esophageal fibrocyte. A strain of liver cell (Fig. 12) of remarkable vigor and reproductive rate was derived from a liver biopsy taken from a 15-month-old infant (No. 6) with undiagnosed disease. The strain has undergone 33 passages in less than 12 months. The parent and two clonal lines closely resemble the liver strain of Chang (1).

DISCUSSION

The history of cells of established strains maintained in continuous culture in this laboratory and of newly derived cell strains has been described. The number of animal cell strains available in many laboratories, primarily used in virus research, has accumulated rapidly since 1952. Propagation of sublines in different laboratories (strain HeLa, for example, has been sent from our laboratory by request to 186 laboratories in all parts of the world) employing various cultural procedures may produce cells with the same name but with differing characteristics. As suggested in the following paper (7), interpretation of experiments with cell strains should not disregard their place of residence. Further, if cell strains are to be useful in studies of cellular biology, the origin should be described as precisely as possible.

Factors affecting the successful growth of cells directly from tissue still are known more by impression than comparative experiment. We have attributed increased frequency of success to use of yeast extract medium and a thin plasma layer for primary culture as well as clonal isolation. Routine use of YEM likewise has eliminated difficulties in cultivation and maintenance of cell strains such as those experienced by Chang (1). A variety of natural (7, 8) and synthetic (2, 3, 6, 9, 17) culture media is rapidly being made available; these are undisputedly valuable for studies in virology and physiology, but possibly should be used cautiously for derivation of cell strains unless extensively tested with many types of cells. Until the selective nature of such media is evaluated, at least one rather than many should be used for cultivation of cells for comparative studies.

SUMMARY

Histories of reception and propagation at the University of Minnesota, and derivation of clonally initiated sublines, are described for HeLa, Maben, liver, and conjunctival human cell strains. Origin and establishment in continuous culture of three new human strains of palate fibrocyte, esophageal epithelial cell, and liver epithelial cell are described. A clonal subline of the esophageal cell has been derived. Success in the establishment of cell strains from human surgical specimens was attributed to use of yeast extract medium and plating of cells in thin layers of plasma.

ACKNOWLEDGMENTS

Important to our work has been the cooperation of Doctors Owen Wangensteen, Donald Crevey, Richard Varco, Robert Good, Fletcher Miller, Logan Leven, William Feller, Paul Lober, and surgical residents in pathology who made it possible to obtain small pieces of operative specimens.

REFERENCES


Figs. 1-6.—Human cell lines in continuous culture. Cells in culture for 4-7 days, stained with Harris hematoxylin and phloxin. Photographs by Mr. Henry Morris. X160.

Fig. 1.—Carcinoma of cervix (HeLa, Gey), parent HeLa cell line I 98, passage 70.

Fig. 2.—Clonal strain of HeLa cell line I 98, passage 7.

Fig. 3.—Pulmonary adenocarcinoma (Maben, Frisch) as clonal cell strain (SC-11).

Fig. 4.—Pulmonary adenocarcinoma (Maben, Frisch) as parent cell line passage 28.

Fig. 5.—Conjunctival cell line (Chang) as clonal strain BA2-A-18.

Fig. 6.—Liver cell (Chang) as clonal strain A1A1-16.
Figs. 7-12.—Human cell lines in continuous culture. Cells in culture for 4-7 days stained with Harris hematoxylin and phloxin. Photographs by Mr. Henry Morris. X160.

Fig. 7.—Normal lung cell (No. 2) line in passage 7.

Fig. 8.—Esophageal fibroblast cell (No. 5) line in passage 18.

Fig. 9.—Esophageal epithelial cell (No. 5) line as clonal strain A1A1-19.

Fig. 10.—Esophageal epithelial cell (No. 5) line as clonal strain A2B14.

Fig. 11.—Palate fibrocyte (No. 3) parent line in passage 13.

Fig. 12.—Liver cell (No. 6) as parent line in passage 32.
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Cancer Res 1957;17:923-926.

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