Classification of Human Colorectal Adenocarcinoma Cell Lines

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SUMMARY

Eleven human colorectal adenocarcinoma cell lines established in this laboratory were classified into three groups based on modal chromosome number, ability to synthesize carcinoembryonic antigen (CEA). Group 1 cell lines contained both dedifferentiated and differentiating cells growing in tight clusters or islands of epithelium-like cells; their modal chromosome number was about 47, and they synthesized small to moderate amounts of CEA. Group 2 cell lines were more dedifferentiated, were hyperdiploid, and synthesized small amounts of CEA. Group 3 cell lines were more differentiated, were hypertriploid, and they synthesized relatively large amounts of CEA. No correlation could be found between Broders' grade or Duke's classification of the original tumor and modal chromosome number or ability to synthesize CEA. These findings support Nowell's hypothesis that the stem line is different for each solid tumor, which makes it difficult to relate chromosomal changes to the initiation of the neoplastic state.

INTRODUCTION

Large-bowel adenocarcinoma represents this country's most common malignant neoplasm, excluding those of skin, and accounts for almost 50,000 deaths yearly (23). No significant change has occurred in the last 30 years in the morbidity or death rate per 100,000 males, and little change has occurred in the last 20 years in the 5-year survival rate after surgical treatment (2). As with most solid tumors, tissue culture has not yet been of great use in the study of these neoplasms because of the paucity of established cell lines (8), and relatively few of those cell lines that have been established have been characterized (5, 18, 25, 26). For tissue culture to be a useful tool, long-term cultures must be established of sufficient strains to reflect the multiple variations of these carcinomas in vivo.

This paper describes the general characteristics of 11 human colorectal cell lines established in this laboratory. On the basis of morphological features, modal chromosome number, and ability to synthesize CEA, these 11 cell lines are divided into 3 groups.

1 To whom requests for reprints should be addressed.
2 The abbreviation used is: CEA, carcinoembryonic antigen.
3 Received June 14, 1976; accepted September 1, 1976.

MATERIALS AND METHODS

Processing of Specimens. All colon, rectal, and involved lymph node tissues studied were from surgical specimens submitted for pathological diagnosis and grading. Grading was by the method of Broders (1); the degree of infiltration or metastasis was indicated by Duke's classification (6).

Upon receipt of the surgical specimen in the pathology laboratory, a tentative diagnosis was made immediately by frozen section. Portions of those specimens known to represent adenocarcinoma were placed in sterile Petri dishes and sent to the tissue culture laboratory. These specimens were immediately covered with a complete growth medium containing antibiotics. Specimens received in the morning were processed the same day. Those received in the afternoon were stored at 4° and processed on the following morning.

Except in the early days of our studies (18), nonenzymatic methods were used in the processing of tumor specimens for tissue culture, namely, the spilbout techniques (14).

Tissue Processing. After removal of normal tissue, necrotic areas, and blood clots from the tumor tissue specimen, it was placed in a sterile plastic Petri dish containing about 15 ml of growth medium. In this dish it was sliced into 1-mm cubes with crossed Bard-Parker No. 11 blades. The supernatant fluid was harvested with a 5-ml pipet fitted with a rubber bulb (Flow Laboratories, Rockville, Md.) and transferred to 15-ml sterile, plastic screw-capped centrifuge tubes (BioQuest, Cockeysville, Md.). The tubes were placed in a rack for 3 min to permit the fine minces to settle. Then, the supernatant was removed to fresh tubas (2nd supernatant).

Tissue minces remaining after the initial removal of supernatant were processed the same day. Those received in the afternoon were stored at 4° and processed on the following morning.

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The 2nd supernatant was centrifuged at 500 x g for 5 min to settle clusters of cancer cells in suspension. This yield was washed at least 3 times with fresh growth medium to remove debris and toxic products being given off by dead or dying cells. After resuspension in growth medium, the viable cells were counted by the trypan blue exclusion technique. This suspension was inoculated into 25-sq cm flasks at 106 viable cells/flask; if less than 106 viable cells were present, the entire yield was inoculated into 1 flask.

The minces remaining after the initial removal of supernatant fluid were transferred to a 50-mi spinner flask (Belco Glass, Vineland, N. J.) in 30 ml of complete growth medium and rotated at 200 rpm for 30 min. The supernatant of this spinner-spilbout technique was processed in the same manner as the 2nd supernatant.
After overnight incubation at 37°, all flasks except those containing fine mince were examined for the presence of viable cancer cells. The supernatants were removed, pooled, and centrifuged at 500 x g for 5 min. The yield was washed 3 times with growth medium and inoculated into a 25-cm² flask in 5 ml of growth medium. The original flasks were refed with 5 ml of medium. The flask containing fine mince were examined after 3 days and fed with 5 ml of growth medium. From then on, all flasks were fed once per week by complete removal of the spent medium and replacement with 5 ml of fresh medium.

**Growth Media.** Our initial growth medium, L-15Cl (15), was Leibovitz L-15 medium (13) supplemented with 10% unheated fetal calf serum (KC Biological Co., Lenexa, Kans.), insulin (Eli Lilly and Co., Indianapolis, Ind.), 0.01 unit/ml; and cortisol (Solu-Cortef; The Upjohn Co., Kalamazoo, Mich.), 10 µg/ml. Additional ingredients were added as detoxifiers, as growth stimulants, or to enable the tumor cells to compete with the more rapidly growing stromal cells. The formula for one such medium, L-15-PAP, has been published (14).

Our most recently developed medium, L-15-D, which is used for detoxification, is described in Table 1. This medium contains catalase (16), methylcellulose (Methocel), and polyvinylpyrrolidone (27) for detoxification; insulin (16), cortisol (11), and Yeastolate for enhancement of growth; heparin (24), sodium polypectate, and sucrose for stabilizing the cell wall; polyestradiol phosphate (Estradurin) to counteract the possible excess of progesterone in fetal calf serum (19); and α-mercapto propionylglycine for its lathyrogen-like action in inhibiting the growth of most collagen-producing stromal fibroblasts. All cultures were maintained in this medium until a cell line was established. Once the cells were adapted to the in vitro environment, this complex medium was no longer required, and they were maintained on medium L-15Cl.

**Passage of Cell Lines.** Cell lines were routinely passed by removing supernatant medium and overlaying the monolayer with 0.25% trypsin (1:250; stabilized for tissue culture):0.1% Versene solution (ICN Nutritional Biochemicals Division, Cleveland, Ohio) in phosphate-buffered saline (per liter: NaCl, 8.0 g; KCl, 0.2 g; KH₂PO₄, 0.12 g; NaH₂PO₄, 0.91 g); pH 7.8. The flask was rotated by hand for 30 sec, decanted, and then placed upside down in a 37° incubator for 5 min. In most instances, the cells flowed from the flask wall; if they did not, the flask was incubated for another 5 min. The cells were harvested by repeated gentle pipetting of 5 ml of growth medium over the monolayer until almost all cells were in suspension. Initially, 1:2 splits were made, but by the 10th passage 1:100 splits were commonly made.

**Generation Time.** Six 25-cm² tissue culture flasks with grid bottoms (Lux flasks; Flow Laboratories) were inoculated with 10⁴ cells in 5 ml of growth medium. The medium was swirled to disperse the cells, and the flasks were incubated at 37°. Two cultures were taken after 24-hr incubation to establish the base-line count. The supernatant fluid was decanted, and the cells were fixed for 5 min in absolute methyl alcohol, stained for 4 min in 1:21 Giemsa stain, rinsed in tap water, and permitted to air dry. A note was made of the cell form (single, doublets, etc.), and 100 consecutive cell counts were made from each flask. The total counts in the 2 flasks (required to agree within 10% for a valid study) were averaged, and this mean was divided by 100 to obtain the base-line count. Two cultures were similarly treated at 7 and at 14 days of incubation. Hayflick’s formula (10) was used to determine the generation time as follows:

\[ n = 3.32 \left( \log N - \log X_0 \right) \]

in which \( n \) = number of generations, \( N \) = final population, and \( X_0 \) = initial population. Multiplication rate, \( r \), or number of generations in a specified time, is calculated by:

\[ r = n / (t_2 - t_0) \]

in which \( t_2 \) = number of time units of study (1 unit = 24 hr) when \( N \) is determined, and \( t_0 \) = time unit at start of study (\( X_0 \) population or 1 unit). The generation time, \( g \), or time for the population to double, is the reciprocal of \( r \):

\[ g = 1 / r \]

**Cyogenetic Studies.** Because our cell lines had relatively long generation times, optimal numbers of cells in mitosis could be obtained by adding the mitotic poison (demecolcine in a final concentration of 0.06 µg/ml) at a time such that overnight incubation at 37° would coincide with an expected division time. The monolayers were dispersed by our trypsin:Versene technique but harvested with 10 ml of hypotonic solution (fetal calf serum:distilled water, 1:5 (v/v)). This cell suspension was transferred to a 15-ml centrifuge tube and incubated at 37° for 30 min. It then was gently
mixed with 5 ml of freshly prepared cold (4°) Carnoy's solution [glacial acetic acid: methanol, 1:3 (v/v)] and incubated at room temperature for 10 min. The cells were harvested by centrifuging at 500 \( \times g \) for 5 min, removing the supernatant, and gently resuspending in 10 ml of fresh Carnoy's solution. The 10-min incubation at room temperature, centrifugation, and resuspension were repeated. After another incubation at room temperature for at least 10 min (the cells can be maintained in this solution overnight if necessary), the cells were again centrifuged at 500 \( \times g \); sufficient supernatant was retained so that, when the cells were resuspended in it, the suspension was hazy.

Clean glass slides were frosted on solid CO₂ and then held at a 45° angle while 3 to 4 drops of the cell suspension were transferred to it and allowed to run down the entire frosted area. The slides were then passed through a flame to ignite the methyl alcohol [the crepe suzette technique of Scherz (22)]. The slide was shaken (as for a fever thermometer) to remove the remaining liquid and allowed to air dry for at least 10 min. Staining was in 1:21 Giemsa for 30 min. If the spreads were not satisfactory, the cells remaining in the tube were resuspended in 10 ml of fresh Carnoy's solution, and the final incubation and centrifugation were repeated.

Thirty-six micrographs of well-spread chromosomes in metaphase were made for each determination of the modal chromosome number. Repeat studies were done with most of the cell lines at higher passage levels to note whether any significant change had occurred in the modal chromosome number.

**Light Microscopy.** All cell lines were stained *in situ* by the hematoxylin-eosin method. The medium was removed from the monolayers growing in 25-sq cm plastic flasks, and the cells were fixed with about 3 ml of absolute alcohol for 5 min. The alcohol was replaced with 5 ml of filtered Harris hematoxylin (Volu-Sol Chemical, Las Vegas, Nev.) for 5 min and then decanted; 5 ml of 0.25% alcoholic eosin Y (Matheson, Coleman and Bell, East Rutherford, N. J.) was added for 1 min and decanted; the stained cells were washed with tap water and allowed to dry in air. Just prior to being photographed, the cells were rehydrated by exposing the layer briefly to a small amount of tap water.

**Electron Microscopy.** Ultrastructure was studied by published methods (18) after the 1st passage, at every 5th passage for the next 25 passages, and then at every 25th passage.

**Screen for CEA Synthesis.** Three 25-sq cm plastic tissue culture flasks were inoculated with 10⁶ cells in 10 ml of L-15 Cl medium and incubated at 37° for 21 days without refeeding. The supernatant from each flask was transferred to a 15-ml centrifuge tube, spun at 500 \( \times g \) for 10 min to remove cells, and then transferred to sterile, screw-capped test tubes for storage at −70° until it could be assayed for CEA. All assays were done within 2 weeks by radioimmunoassay using the Roche kit and procedure manual (Hoffmann-La Roche Inc., Nutley, N. J.). The cells remaining in each flask were harvested by the trypsin-EDTA technique, and the final population of viable cells was determined by the trypsin blue exclusion method. CEA synthesis was calculated per 10⁶ cells to relate our findings to those of other investigators (5).

**Athymic "Nude" Mice.** Inoculation of some of our cell lines into athymic "nude" mice was kindly performed by Dr. B. C. Giovanelli (Stehlin Foundation for Cancer Research, Houston, Texas), Dr. J. Fogh (Sloan-Kettering Institute for Cancer Research, New York, N. Y.), and Dr. Z. Steplewski (Wistar Institute of Anatomy and Biology, Philadelphia, Pa.). The mice were inoculated with 6 × 10⁶ to 1 × 10⁷ cells s.c. into the back or abdomen.

**Glucose-6-phosphate Dehydrogenase Typing.** Typing of the human solid tumor cell lines established in this laboratory was done by Fogh et al. (9). Their technique involved electrophoresis on starch gels (13% starch) in a Tris:EDTA:borate buffer system at pH 8.6 at 5 V/cm for approximately 22 hr at 4°. Then the gels were sliced and stained with a standard tetrazolium staining mixture. The type was determined by comparing the isoenzyme mobilities of test lines with those of control samples of HeLa type A and Detroit 562 type B.

**RESULTS**

**Establishment of Cell Lines.** Of the 163 specimens processed from the middle of 1971 to the end of 1975, 50 were lost to contamination (usually by saprophytic fungi), 88 failed to develop into a cell line, 11 cell lines became established, and 14 are still in progress. The cell line establishment rate increased significantly with the use of complex media (L-15-PAP or L-15-D) designed to neutralize toxic substances released by the dead and dying cells and to enable the tumor cells to grow competitively with the stromal cells (14). A complex medium was no longer required after the cells became established.

The spinner-spillout technique usually yielded 2 to 5 times more cancer cell clusters than did the spillout technique, but these cultures were more grossly contaminated with stromal cells. The supernant harvest pool yielded about as many clusters as did the spinner-spillout supernatant. The fine mince bottles usually showed a mixture of cancer and stromal cells. The use of all 4 methods enhanced the chances of a successful isolation of a permanent cell line.

Viability counts of cancer cells, obtained by the spillout techniques, by the trypsin blue exclusion method ranged from less than 1% to 50%, with the majority of specimens having 10 to 20% viable cells. Although such counts indicated that from about 15,000 to several million viable cells were present (mean, 5 × 10⁶), there was no correlation between cell count and yield of cancer cells capable of proliferating into monolayers. Regardless of the initial count, from 0 to 100 islands of epithelium-like cells were evident (commonly, 10 to 20 in flasks containing viable cancer cells). The cancer cells were readily recognized as tight clusters of islands of epithelium-like cells (Fig. 1); some became firmly attached to the flask wall within 24 hr of explantation, and others were floating in the medium. The clusters of cancer cells appeared to lie dormant for variable periods ranging from 2 weeks to about 6 months before obvious growth was noted.

This long lag phase permitted the stromal cells, which were relatively few in number initially, to proliferate. At first,
the collagenase method of Lasfargues and Moore (12) was used to prevent the fibroblast-like cells from smothering the cancer cells. However, some fibroblast-like cells were not deterred by collagenase, and they completely filled the flask. In one instance, fibroblast-like cells grew under the cancer cell clusters and popped them into the supernatant fluid; the cancer cell clusters were recovered from the supernatant fluid and established as a stroma cell-free cell line (18). Recently, we noted that a mercaptan, \( \alpha \)-mercaptopyrroline (Calbiochem, La Jolla, Calif.), functioned like a lathyrinogen and permitted the cancer cells and stromal cells to grow as cocultures. As with collagenase, this compound does not deter the growth of all fibroblast-like cells. However, the mercaptan also enhanced cancer cell growth and was incorporated at 0.1 \( \mu \)g/ml as part of the deoxification growth medium (L-15-D).

The clusters or islands of epithelium-like cells slowly expanded but, in the initial flasks, would rarely expand sufficiently to form a complete monolayer. Attempts to pass the cells, either by the trypsin-EDTA method or by scraping, before they became well established in their in vitro environment usually were disastrous. When the clusters showed definite evidence of doming, they usually could be passed by either method. One of our lines was maintained in the original flask for more than 1 year before it could be passed successfully.

Some cell lines required at least 12 days for a single generation in early passages (Table 2). As the cells became further adapted to the in vitro environment, the replication rate increased significantly, but replication still required about 2 to 3 days even at high passages. Initially, the monolayers were passed on a 1:2 split, but, usually, by the 10th passage they could be split 1:100, and the daughter cells would again form islands and replicate to form a complete monolayer.

**Grouping of Cell Lines.** The 11 cell lines were placed into 1 of 3 groups based on their cytogenetics, morphological features, and ability to synthesize CEA (Table 3).

These cytogenetic studies were limited to determination of modal chromosome numbers. As noted by Drewinko et al. (5), modern banding techniques would be essential for proper analysis of the karyograms. CEA content in the medium was measured after 21 days of incubation because growth studies in our laboratory had determined that maximal CEA synthesis starts after the cells progress through the log phase of growth and enter the stationary phase. Drewinko and Burk (4) reported similar findings with their LoVo cell line.

Group 1 cells had a modal chromosome number of about 47. By light microscopy, these cells were similar morphologically (Fig. 1) to those reported in the literature (5, 18, 25, 26). Ultrastructurally, 2 distinct cell types were apparent. The cells in the middle of the colonies were isodiametric and loosely arranged with the most notable form of adhesion being desmosomes. Fasciculated filaments were prominent in the cytoplasm. The cells on the periphery of the islands were more columnar and often aligned in a pattern resembling that of normal absorptive epithelium. The free surface of these peripheral cells often formed microvilli. A definite glycocalyx was evident in cell line SW-802 (Fig. 2). CEA synthesis in the Group 1 cell lines was low to moderate, ranging from 8 to 214 ng/10\(^6\) cells.

Both Group 2 cell lines were derived from the same patient. SW-480 was isolated from the primary adenocarcinoma arising in the colon, whereas SW-620 was isolated from a lymph node when the cancer recurred with widespread metastasis. SW-480 cells grew as a mixture of small islands of epithelial cells and individual bipolar cells (Fig. 3). On electron microscopy, the SW-480 cells were polygonal and often had microvilli on their free surfaces (Fig. 4). SW-620 had fewer islands, and most of the cells appeared as a mixture of small individual spherical cells and bipolar cells (Fig. 5). The SW-620 cells seemed to be further dedifferentiated because all cells were isodiametric and there was no evidence of microvilli on their cell surfaces (Fig. 6). Both cell lines were hyperdiploid, and both were low producers of CEA.

The Group 3 cell lines were not significantly different from those of Group 1 by light microscopy. Ultrastructurally, SW-403 and SW-948 had a demonstrable glycocalyx, as did SW-802 (a Group 1 cell line). Two of the Group 3 cell lines, SW-403 and SW-1083, had a prominent Golgi apparatus, which was not noted in any of the other cell lines. SW-837 had unique cytoplasmic structures, resembling stacked lamina, which were often associated with lipid; a thin section of a single cell could contain as many as 5 to 10 of these laminar structures (Fig. 7). Microvesicles were present in all Group 3 cell lines in the cytoplasm and along the brush borders (Fig. 8). Microvesicles have been reported in solid tumor tissue from the surface of neoplastic glandular epithelium, especially in the digestive tract and in mammary glands (3). Two cell lines, SW-837 and SW-1083, had bimodal populations.

### Table 2

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Passage</th>
<th>Generation time (hr)</th>
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<tr>
<td>SW-1116</td>
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* Grouping is based on morphology, cytogenetics, and ability to synthesize CEA.
Grouping of human colorectal adenocarcinoma cell lines by morphology, cytogenetics, and ability to synthesize CEA

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Passage</th>
<th>CEA synthesis (ng/10^6 cells)*</th>
<th>Microvesicular bodies</th>
<th>Chromosome counts*</th>
<th>Tetraploids</th>
<th>Chromosome modal no.</th>
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<tr>
<td>SW-48</td>
<td>63</td>
<td>34</td>
<td>34</td>
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<td>47</td>
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<td>3</td>
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<td>6</td>
<td>55</td>
</tr>
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<td>3</td>
<td>66</td>
</tr>
<tr>
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<td>2500</td>
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<td>5</td>
<td>2</td>
<td>54</td>
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<tr>
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<td>1</td>
<td>42 (85)*</td>
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<td>33</td>
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</table>

* Mean of triplicate analyses by radioimmunoassay (Roche kit) of supernatant from 10^6 cells grown in 10 ml of medium for 3 weeks without refeeding; results corrected for number of cells in final population.

** Distribution of counts by groups of chromosome numbers.

The other cell lines in this group were hyperdiploid to hypotetraploid. All cell lines in Group 3 synthesized relatively large amounts of CEA.

**Correlation with Grade or Classification.** No correlation could be found between either Broders’ grade (1) or Duke’s classification (6) of the original tumor (Table 4) and the modal chromosome number of the cell line or its ability to synthesize CEA. The grades and classifications were randomly dispersed in both Group 1 and Group 3. The Group 2 cell lines, which were the most malignant cell lines, synthesized relatively low amounts of CEA, which also indicated that dedifferentiation and metastasis were not necessarily related to ability to synthesize CEA.

**Special Studies.** To date, SW-48, SW-403, SW-480, SW-620, SW-707, and SW-802 have been injected into athymic nude mice, and successful takes have been obtained (this work was done elsewhere, as noted in "Materials and Methods"). In all instances, the tumors thus produced were consistent histologically with human colon or rectal adenocarcinomas.

Glucose-6-phosphate dehydrogenase typing has been performed with SW-48, SW-480, SW-620, and SW-802, all derived from Caucasian patients. All 4 cell lines were type B.

**DISCUSSION**

In a review of the literature, Rafferty (21) noted the paucity of established human carcinoma cell lines and expressed the fear that most of the widely disseminated cell lines may, in fact, be only one, the HeLa cell. Such fear prompted us to exclude any cell line established in other laboratories and to monitor closely all cell lines established in this laboratory. That our human colorectal adenocarcinoma cell lines were not HeLa cells is demonstrated by their unique morphological and growth characteristics and by the relative constancy of modal chromosome numbers and CEA synthesis over multiple passages, as well as by the results of inoculation into athymic nude mice and glucose-6-phosphate dehydrogenase typing (HeLa cells are phenotype type A).

Establishment of permanent cell lines from human colorectal adenocarcinomas requires isolation of viable clusters of cancer cells from a milieu of dead and dying cells, as well as bacteria and fungi, and protection of these isolated cells from the competitive outgrowth of normal stromal cells while they are adapting to the *in vitro* environment. In our hands, the spillout and spinner-spillout methods have been more satisfactory than trypsinization methods in regard to yield of viable clusters of cancer cells with minimal stromal...
The relatively small yield of viable clusters of cancer cells in relation to the initial count of viable cells (trypan blue exclusion method) suggested that most of the cells were dying and that use of a detoxification medium might enable the surviving cells to withstand the powerful proteases and peroxides released by the dying cells. Although the tumor cells did not grow any better in Medium L-15-D than in Medium L-15-CI, the success rate increased from about 3 to 16% when the medium with detoxification ingredients was used.

To combat contamination, we now use the following antibiotics: gentamicin, 40 μg/ml; streptomycin sulfate, 40 μg/ml; and amphotericin B, 1.25 μg/ml. No evidence of contamination by Mycoplasma organisms has been noted by electron microscopy screening in our laboratory or by a battery of tests in Dr. J. Fogh’s laboratory (personal communication).

The addition of α-mercaptopropionylglycine enabled the cancer cells to compete with the more rapidly growing stromal cells for survival. Often, they grew as cocultures. When the colonies of cancer cells grew sufficiently to start doming (3-dimensional growth), the monolayers could be trypsinized and passed; the original flask was always refed. The cancer cells seemed to be extremely sensitive to trypsinization before they started to grow 3-dimensionally and often would be destroyed if passed prematurely. The stromal cells eventually died out or were overgrown by the cancer cells. The detoxification medium may also be important at this stage to neutralize the toxins released by the dying stromal cells. When the cancer cells were readily subculturable and in relatively pure culture, the complex detoxification medium was no longer required.

Previous investigators (5, 18, 25, 26) have reported that human colorectal adenocarcinoma cells have a modal chromosome number of 47 to 49. These synthesize low to moderate amounts of CEA and resemble our Group 1 cell lines. HT29, isolated by Fogh and Trempe (8), had a biomodal population resembling some of our Group 3 cell lines but synthesized relatively low amounts of CEA (7, 25). This may enable the surviving cells to withstand the powerful proteases and peroxides released by the dying cells.

The cancer cells seemed to be extremely sensitive to trypsinization and whether or not the tumor had metastasized. Our findings support the theory of Nowell (20) that, although abnormalities tend to be more extensive in advanced tumors, a single stem line or clone frequently predominates in all but the early stages. Because the chromosomal alteration characterizing the stem line is different for each solid tumor, this general inconstancy makes it difficult to relate chromosomal changes to the initiation of the neoplastic state.

REFERENCES


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Fig. 1. Tight clusters or islands of epithelium-like cells typical of Group 1 and Group 3 cell lines. H & E, × 350.

Fig. 2. Cells on periphery of islands of epithelium-like cells formed by Group 1 and Group 3 cell lines were often columnar and resembled brush border of normal absorptive epithelium. SW-403, SW-802, and SW-948 had a demonstrable glycocalyx (G). × 10,000.

Fig. 3. SW-480. This Group 2 cell line had loosely bound islands of polygonal, epithelium-like cells and individual spherical to bipolar cells. H & E, × 350.

Fig. 4. SW-480. Electron microscopy revealed epithelium-like cells with large, smooth nuclei. The loosely bound cells often had microvilli on their free surfaces. × 5,000.
Fig. 5. SW-620. This Group 2 cell line formed few small islands of epithelium-like cells and consisted mainly of individual, small, spherical to bipolar-type cells. H & E, × 350.

Fig. 6. SW-620. By electron microscopy, only 1 morphological type of cell was noted. These cells had large nuclei, and their free surfaces were smooth with rare microvilli. × 12,000.

Fig. 7. SW-837. Electron microscopy revealed unique cytoplasmic structures resembling stacked lamina (La) that were often associated with lipid (Li). × 12,000.

Fig. 8. A, Microvesicular bodies (M) were often observed along brush borders of Group 3 cells lines. × 42,000. B, Membrane-bound vesicles (V) containing microvesicular bodies were also found in cytoplasm of Group 3 cell lines. × 14,000.
Classification of Human Colorectal Adenocarcinoma Cell Lines


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