Establishment of Two Parental Cell Lines and Three Clonal Cell Strains from Rat Colonic Carcinoma

Linda S. Borman, Donald C. Swartzendruber, and L. Gayle Littlefield

Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830

ABSTRACT

Two epithelial cell lines were established from separate pools of cells that were harvested by a sequential proteolytic treatment of a single specimen of rat transplantable colonic carcinoma. The cells were small (10-μm diameter) and cuboidal and displayed desmosomes and tight junctions. Both cell lines grew rapidly with population-doubling times of 20 to 22 hr, were near diploid in chromosome number, contained A-2 and B-7 marker chromosomes, and were tumorigenic in rats. Each was distinguishable from the other by the shape of its cell clusters in monolayer culture, its serum requirement for growth, its modal chromosome number, and its karyotypic alterations. Undifferentiated cells, cystic cells, and vacuolated cells, but not mature mucous cells, were observed in monolayer cultures. Clonal cell strains mimicked the morphological and growth properties of their respective parental cell line but display unique karyotypic alterations in addition to the A-2 and B-7 marker chromosomes.

INTRODUCTION

The cell culture of colonic cancer tissue is a relatively new approach to the development of medical therapies and diagnostics, and its potential to provide pure cell populations for study is still under development. Cell lines have been established from human primary colorectal adenocarcinomas (4, 7, 14, 25, 29, 30, 31, 34), from DMH4-induced transplantable tumors of the mouse (2, 28), and most recently from guinea pig colonic adenocarcinoma induced by N-methyl-N-nitrosourea (22). Only one cell line was derived from a particular tumor specimen by each of these investigators, and it generally retained the properties of the parental tumor with respect to the degree of differentiation and heterogeneity of morphology. Cloned cell strains have been isolated from human (4, 5, 30) and mouse (2) cell lines, but only the human DLD-1 cell clones and mouse (4, 5) have been well characterized. The establishment of more than one cell line from a single tumor specimen has been reported; however, in each case, the human cell lines were derived from the primary culture of a single pool of material (25, 29).

The culture of rat transplantable colonic tumor has been reported previously (19), but the properties of the cell lines were not described. We report the culture of cells that were released at different steps in a sequential proteolytic treatment of a single specimen of the rat 4047 transplantable colonic adenocarcinoma (32). Our intention was to fractionate the tumor into crude subpopulations of cells and then to examine the resultant cell lines for distinctive and common biological properties.

MATERIALS AND METHODS

Isolation of Cell Lines. The transplantable colonic carcinoma of the rat, 4047, was developed by Ward et al. (32) from a metastasis of a DMH-induced rat colonic carcinoma. At about monthly intervals, the tumor was passaged by the s.c. injection of a 1-ml aliquot of tumor through a 16-gauge needle into 4- to 6-week-old male Fischer 344 rats. After its ninth passage, the capsulated tumor was surgically excised, washed twice with Saline G (9) supplemented with 105 units of penicillin and 0.5 g of streptomycin sulfate (Grand Island Biological Co.) per liter minced with a pair of surgical scissors, and washed 3 more times with the salt solution. The tumor pieces were subjected to a sequence of enzymatic digestions that consisted of an initial disaggregation of the extracellular matrix followed by a general dissociation of the cell mass: (a) 0.10% collagenase (Nutritional Biochemicals) in Saline G; (b) 0.10% collagenase and 0.05% elastase (type II); Sigma Chemical Co.) in Saline G; (c) 0.05% Pronase (protease from Streptomyces griseus, type VI; Sigma) in calcium- and magnesium-free Saline (d) another Pronase treatment; and (e) 0.10% collagenase in Saline G. Each digestion was carried out in 5.0 ml of enzyme solution at pH 7.0 and with the addition of 1 ml of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (ICN Pharmaceuticals) for 15 min at 36° in a gyratory water bath shaker at 175 rpm. After each digestion, the suspended cellular material and a single wash of the tumor pieces with 1.0 ml of Saline G (with or without dextran cations as required for the next enzyme treatment) was placed in a sterile centrifuge tube (Falcon) that contained 5.0 ml of 4% bovine serum albumin (Fraction V, fatty acid-free; Sigma) in Saline G at 4° and then centrifugation for 8 min at 1000 rpm at room temperature. After a rinse and recentrifugation, the pellet was resuspended in 0.5 ml of Saline G of which 0.1 ml was mixed with 0.1 ml of 1% trypan blue (Microbiological Associates) for a determination of cell number and viability in a hemocytometer, and the remaining suspension was divided equally into two 25-cm flasks (Corning) containing 5 ml of Ham's Medium F12 (8) supplemented with 5% heat-inactivated (56°, 20 min) fetal bovine serum (Sterile Systems) and containing 105 units of penicillin and 0.5 g of streptomycin sulfate per liter. Each culture was given the sequence number (i.e., 1 to 5) of the enzyme solution from which it was derived and the prefix RCC (rat colonic carcinoma). The medium was changed on all cultures 3 times/week. Cells were passaged by rinsing the layer twice with 3.0 ml of calcium- and magnesium-free Saline G and then twice with 2.0 ml of 0.05% trypsin (hog, 1:250; ICN Pharmaceuticals) supplemented with 5 x 10-4 M ethylene bis(oxyethylenetriclio)tetraacetic acid. With a residual film of the trypsin solution left in the flasks, they were capped tightly, placed in a 37° incubator, and shaken intermittently by hand for 15 to 20 min. The nearly detached cell layer was aspirated from the substratum in 1.0 ml of medium and divided 1:2 for the first 6 to 8 weeks of passage and 1:4 thereafter. After 2 months of culture, all cell
lines were maintained in antibiotic-free medium. Periodically, the cell lines, including the cloned cell strains, have been preserved by freezing \((-10^9/\text{min})\) to \(-70^\circ\) in 8% dimethyl sulfoxide in Saline G and then stored in liquid nitrogen. All cell lines, both parental and clonal, are *Mycoplasma* free as judged by electron microscopy.

**Isolation of Cloned Cell Lines.** The cell lines RCC-2 at the fourth passage RCC-5 at the seventh passage were trypsinized from stock flasks, and approximately 400 cells were seeded into a 21-sq cm plate containing 20 to 30 glass chips (2 sq mm) (Bélico). The next day, each glass chip with a single cell attached was transferred to a well in a microtost dish (Falcon). The medium was changed weekly, and approximately 1 month later, each colony of at least 10,000 cells was passaged into an 8-sq cm plate (Falcon), designated as the first clonal passage. Two cloned cell strains were isolated in this way from the RCC-2 (RCC-2-CI-1 and RCC-2-CI-3) and one from the RCC-5 (RCC-5-CI-4) cell lines.

**Transmission Electron Microscopy.** Trypsinized cells were centrifuged at room temperature at 1000 rpm for 8 min and fixed in a modified Karnovsky (12) mixture of 2% paraformaldehyde and 2% glutaraldehyde buffered with 0.1 M sodium cacodylate at a pH of 7.2. The pellet was cut into small pieces after a few min and rinsed in buffer, and one half was postfixed in 2% OsO\textsubscript{4}, all were then dehydrated in acetone and embedded in Epon 812.

Smithin (0.5- to 1-µm) sections affixed to glass slides were stained with methyl blue in a 1% borax solution at pH 11.0 for histochemical study. Nonosmicated cells were treated with the PAS reaction and counterstained with methyl blue.

**Tumorigenicity.** Trypsinized cells were diluted in Saline G and centrifuged at 1000 rpm for 8 min at room temperature. The pellet was resuspended in Saline G, and the cell number was determined with a Coulter Counter or a hemocytometer (Spiers-Levy), containing 2 to 5 x 10\textsuperscript{6} cells/ml. When tumors were of palpable size the colon cancer cell lines since this time. After 2 months of culture in medium with antibiotics, cell lines RCC-3, RCC-4, and RCC-5 were switched to antibiotic-free medium without incident. The RCC-4 line ceased growing and was discarded.

The RCC-2 and RCC-5 cell lines are composed of cuboidal cells that have a diameter of 10 to 15 µm and a large nucleus. Some lines produce confluent cultures of evenly distributed fibroblast-like cells, while other lines produce cultures of compact cell clusters with an outline that is not apparent in the other cell lines at a low magnification and is not shown). RCC-2-CI-3 cells have a distinctive cell morphology. Both RCC-2 and RCC-5 are similar to one another morphologically. Both lines produce compact cell clusters with an outline that is not rounded and globular like RCC-2 but is feathery with sweeping arm-like projections. The glandular-like lumen formed by RCC-5-Cl-4 cells (Fig. 1D) is observed in the other cell lines at a low frequency.

**RESULTS**

Two days after their isolation, a cuboidal cell type predominated in cultures derived from Enzymatic Digestions 2 to 5, but fibroblast-like cells were observed in Culture 1 and to a lesser extent in Culture 2 flasks. Within 6 days, cell growth was observed in all cultures except those established from Treatment 1. Within 1 month of their initiation, Cultures 2 to 5 contained numerous areas of overgrown cuboidal cells and were passaged. All secondary cultures contained very few fibroblast-like cells; however, as a precautionary measure, these were selectively detached from the flask by a 5-min treatment with trypsin solution and discarded. The cuboidal cells were subsequently suspended by trypsinization for 10 min and replated. Fibroblast-like cells have not been seen in the colon cancer cell lines since this time. After 2 months of culture in medium with antibiotics, cell lines RCC-3, RCC-4, and RCC-5 were switched to antibiotic-free medium without incident. The RCC-4 line ceased growing and was discarded.

The parental RCC-2 cells (Fig. 1A) form tight, globular clusters with a rounded, smooth periphery (single arrow) or a diffuse, irregular matrix (double arrows). Each of the clonal cell strains, RCC-2-CI-1 and RCC-2-CI-3, is composed only of diffuse cell clusters of irregular outline (data not shown). RCC-2-CI-3 cells have a distinctive cell morphology at low cell densities, a multicytst configuration that consists of a luminal area that is confined by 2 or 3 cells (Fig. 1B). These structures are lost after several days of growth and are not noticeable in the other colon cancer cell lines. The parental cell line RCC-5 (Fig. 1C) and its clone RCC-5-CI-4 (Fig. 1D) are similar to one another morphologically. Both lines produce cultures of compact cell clusters with an outline that is not rounded and globular like RCC-2 but is feathery with sweeping arm-like projections. The glandular-like lumen formed by RCC-5-Cl-4 cells (Fig. 1D) is observed in the other cell lines at a low frequency. The morphological variety of the colon cancer cell populations is reflected in their ability to achieve confluency in monolayer culture. The cell lines composed of predominantly compact cell clusters, RCC-2 and RCC-5, and its clonal strain, do not grow to cover the substratum, even when they are plated at high cell densities. The cloned strains of RCC-2, on the other hand, produce confluent cultures of evenly distributed cells.

**Histocompatibility Staining.** Staining of the parental cell lines for mucus detected a small amount of PAS-positive material at early passages. However, mature mucous cells (signet ring cells) were not observed in monolayer cultures.
Transmission electron microscopy of the parental cell lines at the second or third passage confirmed their epithelial origin. Small desmosomes were present between cells even after detachment from the substratum (data not shown). At later passages, we observed hemidesmosomes and numerous tight junctions (Figs. 2 and 3). Based upon their ultrastructural properties, several cell types were observed in the parental cell lines that were similar to cells present in the intact (27) and transplantable rat colonic carcinoma (23). These were cystic cells that contained a large intracellular cyst lined with microvilli (Fig. 2), an undifferentiated cell type (Fig. 2), and a cell that displayed a distinct polarity with a tuft of microvilli (Fig. 3). The last cell type contained an eccentric nucleus and numerous vacuoles as do vacuolated cells present in the crypts of a normal colon (3, 13). Mature mucous cells were not observed in any of the cultures. Undifferentiated cells were the most prevalent cell type in all colon cell cultures, parental and clonal. All 5 RCC cell lines were tumorigenic in the host male Fischer 344 rats (data not shown). Palpable tumors (approximately 1 cm in diameter) arose usually within 2 to 3 weeks in animals given injections of 2 to 5 x 10⁶ cells. A striking difference in cellular differentiation was observed between the tumors produced by the RCC-5 cell line and its clone RCC-5-CI-4 (Fig. 4, A and B, respectively). The parental cell line at the 17th passage level produced a tumor with abundant PAS-positive cells uniformly distributed throughout the tumor, whereas the clone at the fifth passage level resulted in a tumor with very few PAS-positive cells. The other parental cell line, RCC-2, produced less-mucinous tumors than RCC-5 as did its clones RCC-CI-1 and RCC-2-CI-3 (data not shown).

The growth of RCC-2 was measured by the cell number and the protein content of cultures (Chart 1). By both criteria, the population-doubling time of this cell line at the 15th or 16th passage was 20 hr. RCC-5 cells doubled every 22 hr at their 30th passage, and the clonal cell lines had population-doubling times of 18 to 20 hr (data not shown). The growth of the colon cancer cell lines is sensitive to the serum concentration of the medium (Table 1). The RCC-2 cell line and its clones grew 50% slower when the serum concentration of the medium was reduced from 5 to 1% serum. However, the same modification of the growth medium of RCC-5 cells and its clone reduced their growth rate at least 3-fold.

The colonic carcinoma cell lines are heterodiploid with a very narrow distribution of chromosome number (Chart 2; 2n R. norvegicus, 42 chromosomes). Whereas the parental RCC-2 cell line contains a majority of cells with 44 chromosomes, both of its clones have a modal chromosome number of 45 chromosomes. Only the RCC-5 cell line and its clone contain cells with a diploid number of chromosomes. Such cells are the major class found in the parental cell line (46%) but represent only 14% of the cells in the cloned cell line which has a modal chromosome number of 43. The incidence of polyploidy in the various cultures ranged from 1.3 to 7.2%.

In addition to variations in modal chromosome numbers between the parental cell lines, each possessed distinctive karyotypic alterations. Analyses of banded and nonbanded karyotypes from RCC-2 and RCC-5 disclosed that each had multiple karyotypic changes including monosomies and trisomies of various chromosomes and several marker chromosomes (Table 2). In the RCC-2 cell line, these included a large supernumerary submetacentric derived from a pericentric inversion of chromosome A-2, a smaller submetacentric appar-

---

**Table 1**

<table>
<thead>
<tr>
<th>Serum concentration (%)</th>
<th>RCC-2</th>
<th>RCC-2-CI-1</th>
<th>RCC-2-CI-3</th>
<th>RCC-5</th>
<th>RCC-5-CI-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>1.05 ± 0.03</td>
<td>1.22 ± 0.03</td>
<td>1.08 ± 0.04</td>
<td>1.46 ± 0.10</td>
<td>1.56 ± 0.05</td>
</tr>
<tr>
<td>1</td>
<td>1.50 ± 0.04</td>
<td>1.48 ± 0.03</td>
<td>1.30 ± 0.04</td>
<td>3.47 ± 0.10</td>
<td>4.91 ± 0.10</td>
</tr>
</tbody>
</table>

*Population-doubling time was determined from the logarithmic portion of the growth curve plot and is expressed as the ratio of the population-doubling time observed for cells grown in medium with various concentrations of serum to those grown in F12FBS5 (control).

*Mean ± S.D. of 2 separate experiments.*
ently derived from a translocation to the short arms of chromosome B-7, and 2 supernumerary D-size metacentrics of unknown origin (Fig. 5). The A-2 and B-7 chromosome markers and one or both small metacentric markers were present in all karyotyped cells of this cell line. The RCC-5 line displayed 8 morphologically distinct marker chromosomes with varying frequencies in 13 karyotyped metaphases. The A-2 chromosome marker was present in all cells, in some instances in lieu of one member of the A-2 chromosome pair, and in other instances as an additional chromosome (Fig. 6). The B-7 chromosome marker was present in 11 cells, while the other 6 markers were observed less frequently (i.e., in from as few as one to as many as 9 of the 13 metaphases), and their derivation has not been determined yet. Analyses of nonbanded material from the 3 clonal cell strains have shown that, while all have some karyotypic changes in common with their parent cell lines (e.g., all clones have A-2 and B-7 chromosome markers), each has unique karyotypic alteration that are different from those observed in the parental cell lines.

**DISCUSSION**

Colonial adenocarcinomas have long been recognized as heterogeneous in histological staining (24). The RCC-2 and RCC-5 cell lines that we isolated from a rat transplantable tumor have morphological, growth, and karyotypic properties that are similar as well as distinctive. In general, they appear similar: they both grow rapidly and are heterodiploid and tumorigenic in isogenic hosts. Also, they display structural and numerical abnormalities of the A-2, B-7, C-13, and D-20 chromosomes that are so alike as to suggest that at some time both cell populations may have evolved from a single progenitor cell. On the other hand, the parental cell lines have different

Table 2

<table>
<thead>
<tr>
<th>Chromosome type no.</th>
<th>A</th>
<th>B</th>
<th>X</th>
<th>Y</th>
<th>C</th>
<th>D</th>
<th>No. of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCC-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 43</td>
<td>+1m</td>
<td>1m</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>1</td>
</tr>
<tr>
<td>2 44</td>
<td>+1m</td>
<td>1m</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>+1</td>
<td>1</td>
</tr>
<tr>
<td>3 44</td>
<td>+1m</td>
<td>1m</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>+1</td>
<td>2</td>
</tr>
<tr>
<td>4 44</td>
<td>1m</td>
<td>+1</td>
<td>1m</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>2</td>
</tr>
<tr>
<td>5 44</td>
<td>+1m</td>
<td>1m</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>+1</td>
<td>2</td>
</tr>
<tr>
<td>6 44</td>
<td>+1m</td>
<td>1m</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>+1</td>
<td>2</td>
</tr>
<tr>
<td>7 45</td>
<td>+1m</td>
<td>1m</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>+1</td>
<td>2</td>
</tr>
<tr>
<td>8 45</td>
<td>+1m</td>
<td>1m</td>
<td>−1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>2</td>
</tr>
<tr>
<td>RCC-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 42</td>
<td>1m</td>
<td>1m</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>3</td>
</tr>
<tr>
<td>2 42</td>
<td>1m</td>
<td>1m</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>2</td>
</tr>
<tr>
<td>3 42</td>
<td>1m</td>
<td>1m</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>1</td>
</tr>
<tr>
<td>4 43</td>
<td>+1m</td>
<td>+1</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>+1</td>
<td>1</td>
</tr>
<tr>
<td>5 43</td>
<td>1m</td>
<td>1m</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>3</td>
</tr>
<tr>
<td>6 43</td>
<td>1m</td>
<td>1m</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>3</td>
</tr>
<tr>
<td>7 44</td>
<td>1m</td>
<td>1m</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>3</td>
</tr>
<tr>
<td>8 44</td>
<td>+1m</td>
<td>1m</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>2</td>
</tr>
<tr>
<td>9 44</td>
<td>+1m</td>
<td>1m</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>2</td>
</tr>
<tr>
<td>10 44</td>
<td>+1m</td>
<td>+1</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>+1</td>
</tr>
<tr>
<td>11 44</td>
<td>+1m</td>
<td>+1</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>+1</td>
</tr>
<tr>
<td>12 44</td>
<td>−1</td>
<td>+1m</td>
<td>1m</td>
<td>+1</td>
<td>1m</td>
<td>−1</td>
<td>1</td>
</tr>
<tr>
<td>13 45</td>
<td>+1m</td>
<td>+1</td>
<td>1m</td>
<td>−1</td>
<td>−1</td>
<td>+1</td>
<td>2</td>
</tr>
</tbody>
</table>

* Chromosomes of unusual size or altered arm ratios whose derivation could not be determined. In RCC-2, 2 supernumerary D-size metacentrics were commonly observed, while 6 distinct marker chromosomes were observed with varying frequencies in 13 karyotypes from RCC-5.
* Karyotypes from banded metaphases.
morphologies and optimal serum requirements for growth in mass culture. In these properties, the clonal cell strains “breed true” to their respective parental type.

These findings are totally consistent with neither a multicellular origin of colonic neoplasia as was suggested by Beutner et al. (1) nor the suspected clonal origin for most human tumors (6). However, the properties exhibited by the RCC-2 and RCC-5 cell lines may have been predicted by the latter theory, which states that tumor progression will occur by the selection of variant cell strains that arise with time from the genetically unstable malignant cell population (21). We cannot say that the RCC-2 and RCC-5 cell populations, as we have characterized them in vitro, existed as such in the original tumor. However, both cell lines were treated identically since isolation and morphological differences which were apparent from early passages have remained stable for over 1 year in culture.

In conclusion, a number of general but noteworthy points should be made about this rat colonic adenocarcinoma cell system. (a) The presence of chromosome A-2 abnormalities in cell lines from a DMH-induced tumor is similar to those described in sarcomas, carcinomas, and leukemias induced in rats treated with several other polycyclic hydrocarbons including 7,12-dimethylbenz(a)anthracene, 20-methylcholanthrene, and 3,4-benzopyrene (see Refs. 16, 17, and 20 for review). (b) The ability of RCC-5 cells to produce highly mucinous tumors in rats but not in monolayer culture offers an experimental system for the study of the hormonal, nutritional, and/or physical conditions necessary for the cytodifferentiation of colon cells. And (c) we are not acquainted with any reports of cystic and/or vacuolated cells in cultures derived from human or mouse colonic adenocarcinoma. The latter cells are present in the crypts of the normal colon and have been postulated to be the crypts of the normal colon and have been postulated to be

ACKNOWLEDGMENTS

We thank Pat Miller for her assistance in the transmission electron microscopy study and Anne Sayer for her contribution to the cytogenetics analyses.

REFERENCES

Fig. 1. Light micrographs of cultures of rat colon cancer cell lines. Giemsa stained *in situ*, × 350. A, RCC-2 cells at the 11th passage. Compact, globular cell clusters are characteristic of this cell line (single arrow); disperse cells are also present (double arrows). B, RCC-2-CI-3 cells at the third clonal passage 1 day after seeding the cultures. Note the cyst-like structures enclosed by as few as 2 cells (c). C, RCC-5 cell line at the 20th passage. Cells are clustered into irregular shapes with smooth outlines. D, RCC-5-CI-4 at the third clonal passage. A glandular-like lumen has been formed by 10 to 15 cells (l).
Fig. 2. Electron micrograph of RCC-5 cells at the 20th passage. Note the large intracytoplasmic cyst lined with microvilli (c). Tight junctions (t) are present between cells, and several undifferentiated cells (u) are observed. × 10,000.

Fig. 3. Electron micrograph of RCC-2 cells at the 12th passage. Tight junctions (t) are present between adjacent cells. A tuft of microvilli (m) and numerous vacuoles lined with microvilli-like projections (v) are noted. × 16,000.
Fig. 4. Light microscopic photographs of sections of tumors produced in male Fischer 344 rats by the colon cancer cell lines. Note the abundant PAS-positive cells in the tumor that arose from RCC-5 cells (A) and the paucity of similar cells in the tumor derived from the cloned strain RCC-5-CI-4 cells (B). PAS stain, × 150.
Fig. 5. Examples of 2 banded metaphase karyotypes from RCC-2 (13th passage) cells. 45 chromosomes in upper field and 44 chromosomes in lower field. In addition to monosomies and trisomies of various chromosomes, note the large supernumerary submetacentric marker derived from a pericentric inversion of an A-2 chromosome and the small metacentric marker apparently derived from a translocation to the short arms of chromosome B-7. One or 2 additional D-size metacentrics of unknown origin (right side of karyotype) were present in all karyotyped metaphases from this cell line.
Fig. 6. Karyotypes of 2 nonbanded metaphases from RCC-5 (22nd passage) cells, 44 chromosomes in both the upper and lower fields. Eight morphologically distinct markers were observed with varying frequencies in 15 karyotyped metaphases from this cell line. Examples of markers in addition to A-2 and B-7 are shown on the right side of the karyotypes.
Establishment of Two Parental Cell Lines and Three Clonal Cell Strains from Rat Colonic Carcinoma

Linda S. Borman, Donald C. Swartzendruber and L. Gayle Littlefield


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/12/5074

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.