Characteristics of Cell Lines Established from Human Colorectal Carcinoma

Jae-Gahb Park,1 Herbert K. Oie, Paul H. Sugarbaker,3 Jerry G. Henslee, Tchaw-Ren Chen, Bruce E. Johnson, and Adi Gazdar

NCI-Navy Medical Oncology Branch, National Cancer Institute and Naval Hospital, Bethesda 20814 [J-G. P., H. K. O., B. E. J., A. G.]; Surgery Branch, National Cancer Institute, Bethesda, Maryland 20892 [P. H. S.]; Abbott Laboratories, North Chicago, Illinois 60064 [J. G. H.]; and American Type Culture Collection, Rockville, Maryland 20853 [T-R. C.]

ABSTRACT

We have characterized 14 human colorectal carcinoma cell lines established from primary and metastatic sites by us during the years 1982 to 1985. Five lines were established in fully defined ACL-4 medium and 9 in serum supplemented R10 medium. However, after establishment, cultures could be grown interchangeably in either medium. The lines grew as floating cell aggregates in ACL-4 medium, while most demonstrated substrate adherence in R10 medium. The lines had relatively long doubling times and low cloning efficiencies. Twelve were tumorigenic in athymic nude mice when injected s.c., and two grew i.p. as well. Based on culture, xenograft, and ultrastructural morphologies, the 14 lines could be subtyped as follows: 4 were well differentiated; 5 were moderately differentiated; 4 were poorly differentiated; and 1 was a mucinous carcinoma. Membrane associated antigens characteristic for gastrointestinal cells (carcinoembryonic antigen, CA 19-9, and TAG-72 antigens) were expressed by 50-71% of the lines. Lines expressing carcinoembryonic antigen and CA 19-9 actively secreted these antigens into the supernatant fluids while TAG-72 antigen was not secreted. Surprisingly, 5 of 7 of the original tumor samples tested and 13 of 14 cultured lines expressed L-dopa decarboxylase activity, which is a characteristic enzyme marker of neuroendocrine cells and tumors. In addition, one poorly differentiated cell line contained dense core granules, characteristic of endocrine secretion. Preliminary cytogenetic analyses indicated that 9 of 11 lines examined contained double minute chromosomes. In addition, 3 of the 9 lines with double minute chromosomes had homogeneously staining regions. These findings indicate a high incidence of amplification of one or more as yet unidentified genes.

INTRODUCTION

Colorectal cancer is one of the commonest solid tumors, and its incidence is second only to lung cancer in the United States. An estimated 140,000 new cases will be diagnosed in 1986, 60,000 of whom will die of their disease (1). Cell lines established from human colorectal carcinomas may provide useful tools to study the biology of this disease and to develop and test new therapeutic approaches. A relatively large pool of well characterized cell lines already exists. At least three other groups have established multiple colorectal cell lines (2-4), and several other reports describe the establishment of one or two lines (for review, see Ref. 4). Also, colonic adenomas have been successfully cultured recently (5). A variety of culture techniques have been employed, including the use of feeder layers (3), enriched serum containing media (4), and the use of collagen gels (5).

A large bank of well characterized cell lines may reflect the diversity of tumor phenotypes and provide adequate models for studying tumor heterogeneity. Thus, cell lines with unusual or unique characteristics continue to be described (5, 6). Our laboratory has extensive experience with the use of serum containing as well as fully defined media for the establishment of continuous cell lines derived from lung cancers (7). We have utilized this experience to establish and characterize 14 cell lines of colorectal carcinoma origin. Some of the properties of our cell lines, including expression of the NE4 cell associated enzyme DDC and cytogenetic evidence for frequent gene amplification have not been described previously.

MATERIALS AND METHODS

Cell Culture. Cell lines were established from pathologically proven colorectal tumors, either directly or after heterotransplantation in athymic nude mice. Solids tumors were finely minced with scissors and dissociated into small aggregates by pipetting. For culture of primary tumors invasive areas were used whenever possible. If tumor from the mucosal surface had to be used, the surface was cleansed with 70% ethanol or iodine containing solutions. Ascitic fluids were collected, pelleted, washed, and resuspended in growth medium. Approximately 1-5 x 10^6 cells were seeded into 25-cm^2 flasks. Tumors were cultured in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum (R10), or in ACL-4 medium, a fully defined medium formulated for the selective growth of human lung adenocarcinoma (8, 9). Initial cell passage was performed when heavy tumor cell growth was observed (usually 2-4 weeks after initial culture). Subsequent passages were performed weekly. Nonadherent cultures were passed by transfer of floating multicellular aggregates. Adherent cultures were passaged at subconfluence after trypsinization. If stromal cell growth was noted in initial cultures, differential trypsinization (3) was used to obtain a pure tumor cell population. Media and sera were obtained from Grand Island Biological Co., Grand Island, NY. Cultures were maintained in humidified incubators at 37°C in an atmosphere of 5% CO_2 and 95% air. The sources of the additives used in ACL-4 medium are listed in Ref. 9. Culture morphology was observed by phase contrast microscopy.

Growth Characteristics. Population doubling times were determined by seeding 3 x 10^4 viable cells into replicate 25-cm^2 flasks and performing counts every 3 days for 4 weeks or longer. Cultures were fed every 3 or 4 days and 24 h prior to counting. For plating efficiencies, 10^5 cells were plated in 5 replicate 100-mm dishes, and colonies consisting of more than 50 cells were enumerated 21-30 days later, after staining with 0.5% crystal violet.

For determination of colony forming efficiency in semisolid medium, 10^4 viable single cells were plated in 3 ml of R10 medium containing 0.3% agarose over a base layer of R10 medium containing 0.5% agarose in 5 duplicate 60-mm dishes.

Cell Culture Characterization. Tumorigenicity was tested by inoculating 5-10 x 10^6 cells s.c. into each of 5 male athymic nude mice, BALB/c background (Frederick Cancer Research Center, Frederick, MD) and observing twice weekly for progressive growth. Histological examination was performed on all tumors so obtained. For some cell lines, 1 x 10^7 tumor cells were inoculated i.p. as well in other groups of athymic mice.

Ethanol fixed monolayer cultures grown on glass slides, Saccomanno fluid fixed cytoospin preparations of floating cultures, and formalin fixed

Received 12/29/86; revised 7/24/87; accepted 9/8/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The opinion or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy or the Department of Defense.

2 To whom requests for reprints should be addressed, at Department of Surgery, College of Medicine, Seoul National University and Seoul National University Hospital, Seoul 110, Korea.

3 Present address. Department of Surgery, Emory University, School of Medicine, Atlanta, GA 30322.

4 The abbreviations used are: NE, neuroendocrine; DDC, L-dopa decarboxylase; CEA, carcinoembryonic antigen; DM, double minute; HSR, homogeneously staining region.
paraffin embedded sections of xenografts were stained with hematoxylin-eosin, alcian blue, and mucicarmine.

For ultrastructural studies, cell pellets were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, stained with 1% uranyl acetate, and thin sections were examined by a Siemens 1A electron microscope.

Cytogenetic studies were performed in 11 cell lines: NCI-H498, NCI-H508, NCI-H548, NCI-H630, NCI-H684, NCI-H716, NCI-H747, SNU-C1, SNU-C2A, SNU-C4, and SNU-C5. Chromosome slides were prepared by standard air-dry method from exponentially growing cultures after 45- to 60-min Colcemid treatment (0.1 μg/ml final concentration) and stained with Giemsa. Modal chromosome number and occurrence of double minutes were evaluated from 50 metaphase spreads. The rate of polyploidy was evaluated by scanning 500 metaphases.

Aromatic amino acid decarboxylase (EC 4.1.1.28), also known as L-dopa decarboxylase, was assayed by our modification (10) of a reported method (11).

Mycoplasma contamination was tested for by direct agar isolation and Hoechst stain methods by Microbiological Associates, Inc. (Bethesda, MD), and confirmed by us with the use of a ribosomal RNA hybridization method (Gen-Probe, San Diego, CA).

All lines were tested for murine virus contamination by the mouse antibody production tests (12).

Cell homogenates were tested for the human forms of the following enzymes by starch gel electrophoresis (13), using the Authentikit system (Corning Science Products, East Walpole, MA); purine nucleoside phosphorylase (EC 2.4.2.1); glucose-6-phosphate dehydrogenase (EC 1.1.1.49); peptidase B (EC 3.4.11.4); and lactate dehydrogenase (EC 1.1.1.27).

Antigen Expression and Secretion. Expression and secretion of CEA (14), CA 19-9 (15) and TAG-72 (16–18) surface antigens were measured by sandwich immunoassay using a CA 19-9 monoclonal antibody (Abbott Laboratories, North Chicago, IL), and confirmed by us with the use of a homologous sandwich radioimmunoassay using the B72.3 monoclonal antibody in a microtiter plate assay configuration.

RESULTS

Culture Characteristics. Fourteen colorectal carcinoma lines were established from 6 primary and 8 metastatic tumors. Nine of the lines were from patients who had not received prior cytotoxic therapy, while 5 lines were from patients who had previously received cytotoxic therapy including 5-fluorouracil. Relevant clinical information is provided in Table 1. Eleven of the tumors were cultured directly, while 3 were established from xenografts.

Nine lines were established in R10 and five in ACL-4 medium (Table 2). In order to compare establishment rates, 24 tumor specimens were cultured in both media. Six cell lines were established in R10 medium (25%), while 9 were established in ACL-4 medium (38%) and included all 6 specimens successfully cultured in R10. The minimum number of viable tumor cells necessary for successful culture in either medium was estimated to be 5–10 × 10^5. In both media growth occurred either immediately or after a dormant period lasting up to eight weeks.

Once cell growth commenced, it tended to be progressive, without periods of acute cell death ("crises"). After establishment, all lines could be grown in either medium. For uniformity, cell line characterization as described herein was performed on cultures in R10 medium unless stated otherwise. All lines, including those initiated from xenografts, expressed human forms of the 4 enzymes tested and were free of contamination with Mycoplasma or murine viruses.

Twelve of the 14 lines were tumorigenic in athymic nude mice (Table 2). Population doubling times in R10 medium ranged from 34 to 94 h. Plating efficiencies and colony formation in soft agarose in R10 medium were relatively modest (Table 2).

Morphological Studies. In R10 medium, most cultures grew as adherent monolayers (Table 2). One culture, SNU-C1, displayed both adherent and floating subpopulations. Cell line NCI-H498 grew as floating cell aggregates surrounded by a halo of easily visualized mucinous material. If the mucin coat was removed and the cells dispersed by trypsinization, the line could be adapted to a monolayer culture. Cell line NCI-H716 completely lacked substrate adherence and grew as amorphous floating cell aggregates.

In ACL-4, which lacks attachment factors, 12 of 14 cultures lacked substrate adherence and grew as floating gland-like structures or amorphous aggregates. Most of the floating cultures could be grown as monolayers in ACL-4 medium if the culture dishes were precoated with collagen (Vitrogen 100, Collagen Corporation, Palo Alto, CA).

Based on culture, xenograft, and ultrastructural morphology, cultures could be subdivided into 4 subtypes: 4 were well differentiated; 5 were moderately differentiated; 4 were poorly or undifferentiated; and 1 was a mucinous carcinoma. Culture morphology was most accurately determined in ACL-4 medium, although some features could also be discerned in R10 medium. Dome formation could only be evaluated in confluent monolayers grown in R10 medium. The salient features of each subtype are described below, and summarized in Table 2.

Well Differentiated Cell Lines. In ACL-4 medium, well differentiated cultures grew as floating cell aggregates, sometimes as acinar structures surrounding central lumenini. A characteristic feature was the uniform, radial orientation of tall columnar cells (Fig. 1A). In R10 medium, they consisted of relatively large epithelioid cells. Prominent dome formation (19) was present at confluence in two of the cell lines (Fig. 1B). In addition to dome formation, three dimensional gland-like structures were present in confluent adherent cultures. Corresponding xenografts demonstrated well formed glands lined by tall columnar cells having prominent apical borders (Fig. 1C). The most characteristic ultrastructural features were uniform microvilli with prominent filamentous core rootlets and glycoalyceal bodies (Fig. 1D), as well as well formed junctional complexes.

Moderately Differentiated Cell Lines. Cell lines with features intermediate between, and sometimes overlapping with the well and poorly differentiated subtypes were arbitrarily classified as moderately differentiated (Fig. 2). In ACL-4 medium the cells formed spheroid or irregular masses rather than gland-like structures, and in R10 medium dome formation, if present, was inconspicuous. The cells tended to be cuboidal rather than columnar, and gland formation was less prominent. Microvilli were less uniform in size, and filamentous core rootlets, glycoalyceal bodies, and well formed junctional complexes were only occasionally present.

Poorly Differentiated Cell Lines. Poorly differentiated cultures grew in ACL-4 medium as single cells or small amorphous...
aggregates. In R10 medium they displayed varying degrees of substrate adherence, but line NCI-H716 did not attach at all (Fig. 3A). No evidence of gland or dome formation was observed. Xenograft histology consisted of sheets of undifferentiated cells with or without feeble attempts at gland and mucin formation. No characteristic ultrastructural features were seen, except in cell line NCI-H716, which contained moderate numbers of spherical cytoplasmic granules, about 300 nm in diameter, containing electron dense centers, surrounded by narrow lucent halos and bound by a unit membrane (Fig. 3B). Xenografts of the NCI-H716 cell line had additional unusual features; s.c. tumors frequently invaded and destroyed surrounding tissues; s.c. tumors frequently invaded and destroyed surrounding tissues; s.c. tumors frequently invaded and destroyed surrounding tissues. However, prominent extracellular mucin secretion was a feature only present in NCI-H498 cells. The aggregates would not attach to the substrate in R10 medium unless the mucinous coat was dispersed by trypsinization. Monolayer cells were large and often distended with mucin droplets (Fig. 4B). Gland and dome formation were occasionally present. Xenograft histology and ultrastructural morphology indicated that the cell line had characteristics of a well differentiated adenocarcinoma, and confirmed the prominent mucin secretion.

An additional feature of interest was the ability of the cell line to form tumors after i.p. inoculation (Fig. 4C). The tumor cells formed floating cell aggregates with gland formation surrounded by mucinous coats, closely mimicking their cultural appearance in ACL-4 medium. Spread to the right pleural cavity was frequently noted (Fig. 4D).

Expression and Secretion of Antigens. We studied colorectal cell line expression and secretion of antigens associated with gastrointestinal cells and their tumors. As demonstrated in Fig. 5, of the 14 lines, TAG-72, CA19-9, and CEA were expressed with carcinoid features. Its mucinous nature. Mucin stains indicated varying degrees of intracellular mucin production by other cell lines and their corresponding xenografts, especially the well differentiated lines. However, prominent extracellular mucin secretion was a feature only present in NCI-H498 cells. The aggregates would not attach to the substrate in R10 medium unless the mucinous coat was dispersed by trypsinization. Monolayer cells were large and often distended with mucin droplets (Fig. 4B). Gland and dome formation were occasionally present. Xenograft histology and ultrastructural morphology indicated that the cell line had characteristics of a well differentiated adenocarcinoma, and confirmed the prominent mucin secretion.

An additional feature of interest was the ability of the cell line to form tumors after i.p. inoculation (Fig. 4C). The tumor cells formed floating cell aggregates with gland formation surrounded by mucinous coats, closely mimicking their cultural appearance in ACL-4 medium. Spread to the right pleural cavity was frequently noted (Fig. 4D).

Expression and Secretion of Antigens. We studied colorectal cell line expression and secretion of antigens associated with gastrointestinal cells and their tumors. As demonstrated in Fig. 5, of the 14 lines, TAG-72, CA19-9, and CEA were expressed.

### Table 1 Origin of colorectal carcinoma cell lines

<table>
<thead>
<tr>
<th>Degree of differentiation</th>
<th>Date of initiation</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Blood type</th>
<th>Prior therapy</th>
<th>Primary tumor site</th>
<th>Cultured tumor site</th>
<th>Original tumor differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>NCI-H548</td>
<td>1/83</td>
<td>M</td>
<td>W</td>
<td>A+</td>
<td>None</td>
<td>Sigmond</td>
<td>Primary</td>
<td>Well, with carcinoid features</td>
</tr>
<tr>
<td>Moderately</td>
<td>NCI-H630</td>
<td>9/83</td>
<td>M</td>
<td>W</td>
<td>A+</td>
<td>FAM and R</td>
<td>Rectum</td>
<td>Liver</td>
<td>Moderately</td>
</tr>
<tr>
<td>Poorly/undifferentiated</td>
<td>NCI-H742</td>
<td>6/84</td>
<td>M</td>
<td>W</td>
<td>A+</td>
<td>5-FU</td>
<td>Cecum</td>
<td>Primary</td>
<td>Moderately</td>
</tr>
<tr>
<td>Mucinous</td>
<td>NCI-H716</td>
<td>7/84</td>
<td>M</td>
<td>W</td>
<td>A+</td>
<td>None</td>
<td>Cecum</td>
<td>Moderately</td>
<td>Well</td>
</tr>
<tr>
<td></td>
<td>SNL-C1</td>
<td>8/84</td>
<td>M</td>
<td>W</td>
<td>O+</td>
<td>None</td>
<td>Descending</td>
<td>Peritoneum</td>
<td>Moderately</td>
</tr>
</tbody>
</table>

### Table 2 Culture morphology and growth characteristics of colorectal cell lines

Cultures were initiated in serum supplemented R10 or fully defined ACL-4 media. After establishment, sublines were cultured in both media. Cell lines were evaluated for presence or absence of substrate adherence in both media. Morphological features of differentiation (G, gland like structures; D, dome formation; ECM, extracellular mucin secretion; U, undifferentiated) were evaluated in both media and are presented as a composite. ND, not done; F, not done due to lack of substrate adherence.

<table>
<thead>
<tr>
<th>Degree of differentiation</th>
<th>Initial growth medium</th>
<th>Substrate adherence</th>
<th>Culture appearance</th>
<th>Doubling time (h)</th>
<th>Plating efficiency (%)</th>
<th>Cloning efficiency (colonies/10⁹ cells)</th>
<th>Tumorigenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>NCI-H548</td>
<td>R10</td>
<td>–</td>
<td>73</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NCI-H630</td>
<td>R10</td>
<td>+</td>
<td>46</td>
<td>3.5</td>
<td>800</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NCI-H684</td>
<td>ACL-4</td>
<td>+</td>
<td>74</td>
<td>2.7</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NCI-H958</td>
<td>ACL-4</td>
<td>–</td>
<td>110</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Moderately</td>
<td>NCI-H508</td>
<td>R10</td>
<td>–</td>
<td>53</td>
<td>5.6</td>
<td>8</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NCI-H742</td>
<td>ACL-4</td>
<td>+</td>
<td>ND</td>
<td>1.0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>NCI-H747</td>
<td>ACL-4</td>
<td>±</td>
<td>65</td>
<td>5.1</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NCI-H768</td>
<td>ACL-4</td>
<td>±</td>
<td>50</td>
<td>0.8</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNL-C1</td>
<td>R10</td>
<td>±</td>
<td>45</td>
<td>F</td>
<td>1300</td>
<td>+</td>
</tr>
<tr>
<td>Poorly</td>
<td>SNL-C2A*</td>
<td>R10</td>
<td>+</td>
<td>82</td>
<td>0.5</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNL-C4</td>
<td>R10</td>
<td>+</td>
<td>34</td>
<td>6.5</td>
<td>310</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNL-C5</td>
<td>R10</td>
<td>+</td>
<td>67</td>
<td>3.3</td>
<td>380</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNL-H716</td>
<td>R10</td>
<td>–</td>
<td>67</td>
<td>F</td>
<td>2500</td>
<td>+</td>
</tr>
<tr>
<td>Mucinous</td>
<td>SNL-H498</td>
<td>R10</td>
<td>+</td>
<td>94</td>
<td>0.2</td>
<td>5</td>
<td>+</td>
</tr>
</tbody>
</table>

* After trypsinization and removal of the surrounding mucinous coat.
CHARACTERISTICS OF COLORECTAL CARCINOMA CELL LINES

Fig. 2. Morphology of moderately differentiated lines. A, phase contrast photomicrograph of cell line NCI-H508 in ACL-4 medium. The floating cell aggregates are attempting to form glandular structures. B, xenograft of cell line NCI-H508 demonstrating occasional gland-like spaces lined by cuboidal cells.

by 7, 9, and 10 lines, respectively. Five lines expressed all 3 antigens, while 2 lines expressed none of the 3 antigens. There was no apparent relationship between antigen expression and the morphological phenotype. With one exception, all lines expressing CA19-9 and CEA secreted the antigens into the supernatant fluids. There was an excellent correlation between the intracellular and secreted antigen levels. In contrast, cell lines expressing TAG-72 antigen failed to secrete the antigen (Fig. 5).

Expression of DDC. We examined our 14 cell lines and 7 of the tumors from which they were initiated for expression of DDC, an enzyme characteristic of NE cells (14). Enzyme activity (>1 unit/mg protein; 1 unit = 1 nM CU2/h) was detected in 13 of 14 lines and 5 of 7 tumors (Table 3). Relatively high levels (>10 units) were present in 10 of 14 lines and 3 of 7 tumors. There was a tendency for enzyme levels to rise during establishment and passage of the cell lines (Fig. 6), and in one cell line (NCI-H716) relatively high levels were expressed by the line while the tumor lacked detectable activity (Fig. 6).

DDC levels were correlated with the presence of cytoplasmic dense core ("neurosecretory") granules. Only one cell line, NCI-H716, expressed many relatively large (300 nm) dense core

Fig. 1. Morphology of well differentiated cultures. A, phase contrast photomicrograph of cell line NCI-H548 in ACL-4 medium demonstrating partial substrate attachment (lighter areas). The floating component consists of tubuloglandular structures lined by tall columnar cells. B, cell line NCI-H548 in RPMI 10 medium, demonstrating attached epithelioid morphology and prominent dome formation; C, xenograft of cell line NCI-H548 demonstrating well formed glands; D, electron micrograph of cell line NCI-H548 demonstrating junctional complex (jc), microvilli (m), filamentous core rootlets (fr), and glycocalyx bodies (gb).
granules. Granules were detected in all of the cells examined.

Cytogenetic Studies. Cytogenetic studies were performed on 11 of 14 cell lines (chromosome studies on cell lines NCI-H742, NCI-H768, and NCI-H958 have not been completed). One of the lines examined was pseudodiploid, while 10 were aneuploid with modal chromosome numbers varying from 47 to 102 (Table 4). One culture (SNU-C1) had a modal chromosome number of 77–78 for 103 passages over a period of 18 months, indicating its relative stability. Surprisingly, 9 of 11 of the lines examined had DM chromosomes. In 3 of these lines (NCI-H684, SNU-C2A, and SNU-C5), the DMs occurred mostly at 1/metaphase. In the remaining 6 positive lines, DM positive metaphases were easily detected and the number of DMs per metaphase varied from 2 to over 200. Cell line NCI-H508 contained DMs of two sizes, those of typical size, and considerably larger ones which we term chromat blocks (Fig. 7). In addition, cell lines NCI-H508, NCI-H630, and NCI-H716 contained HSRs. More detailed karyological analyses will be presented in a separate report.

DISCUSSION

In this report we describe the establishment and extensive characterization of 14 continuous cell lines derived from human primary and metastatic colorectal carcinomas. The culture success rate in ACL-4 medium (38%) was greater than that in serum supplemented R10 medium (25%).

Multiple colorectal cell lines have been established by others (2-6). However, our report describes several interesting or unique features, including: (a) the use of a serum-free medium for the establishment and maintenance of colorectal lines; (b) expression and secretion of high concentrations of 3 gastrointestinal cell associated antigens; (c) possibly unique phenotypes (mucinous carcinoma and dense core granule containing lines); (d) high frequency of expression of the NE cell marker DDC; and (e) high frequency of cytogenetic evidence for gene amplification. Of interest, 3 of the cell lines reported herein were established from xenografts by one of us (J-G. P.) in Korea (Seoul National University series) and had morphologies and properties similar to lines established directly from tumors in the United States (by H. K. O. and A. F. G.) (National Cancer Institute series). Other than substrate adherence, lines established or adapted to serum-containing medium or serum free medium had similar properties.

Five of the 14 lines were established and maintained in ACL-4, a fully defined medium originally formulated for the selective growth of human adenocarcinoma of the lung (7-9). While ACL-4 has proved useful in establishing many miscellaneous types of human cancers (9), certain human tumors, such as small cell lung cancer, fail to propagate efficiently in ACL-4.
One of the advantages of using fully defined media (including ACL-4) for the selective growth of the tumors is the failure of stromal cells to replicate for very long under such conditions. After establishment, all of the lines could be grown in either medium. Van der Bosch et al. (20) have described a defined medium (somewhat similar to ACL-4) for the culture of colon tumors; however, most of their cultures only grew for short periods.

Cell lines maintained in R10 medium usually demonstrated substrate attachment, while those grown in ACL-4 usually lacked it unless the culture dishes were precoated with collagen.

---

**Table 3** Dopa decarboxylase concentrations in colorectal cell lines

<table>
<thead>
<tr>
<th>Degree of differentiation</th>
<th>DDC concentration (units/mg protein)</th>
<th>Dense core granules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumor</td>
<td>Cell line</td>
</tr>
<tr>
<td>Well</td>
<td>NCI-H548</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>NCI-H630</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>NCI-H684</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>NCI-H958</td>
<td>53</td>
</tr>
<tr>
<td>Moderately</td>
<td>NCI-H508</td>
<td>NT*</td>
</tr>
<tr>
<td></td>
<td>NCI-H742</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>NCI-H747</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>NCI-H768</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>SNU-C1</td>
<td>NT</td>
</tr>
<tr>
<td>Poorly</td>
<td>SNU-C2A</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>SNU-C4</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>SNU-C5</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>NCI-H716</td>
<td>NT</td>
</tr>
<tr>
<td>Mucinous</td>
<td>NCI-H498</td>
<td>NT</td>
</tr>
</tbody>
</table>

* NT, not tested.
overlapping with the other subtypes. One possibly unique cell line, NCI-H716, may constitute another possibly unique colorectal subtype was abitrary, as these lines often had features that differentiated well and poorly differentiated lines (24, 25). CAI9-9, originally identified from a colon carcinoma line, is a sialylated lacto-Af-fucopentaose II, an oligosaccharide related to Lewis blood group substances (9, 26–29). Elevated levels of the CA 19-9 epitope are present in the sera of patients with colonic and other gastrointestinal cancers (30, 31). TAG-72, a high molecular weight glycoprotein, is expressed by many epithelial malignancies including colon, breast, and ovary (32). Its expression appears to be influenced by the spatial configuration of the tumor cells, and it is reported to be rarely expressed in monolayer cell cultures (33). These three antigens were expressed by 50–71% of the lines, and there was little correlation with degree of differentiation. Lines expressing CEA and CA 19-9 actively secreted these antigens into the supernatant fluids, while TAG-72 antigen was not secreted. While elevated levels of the TAG-72 antigen were detected in the sera of some patients with metastatic colon carcinomas (34), our findings suggest that CEA and CA 19-9 may function as more sensitive serum markers than TAG-72.

Many (13 of 14) of our cell lines expressed varying concentrations of DDC, a key NE cell marker (35). DDC is essential for formation of biogenic amines, both serotonin and catecholamines. Five of 7 tumor specimens examined from which the cell lines were derived expressed DDC. The incidence and concentrations of DDC increased after in vitro growth. These data are in agreement with a large survey of human tumors that indicated that non-small cell lung cancers (12%) and colorectal tumors (51%) were the only non-NE tumor types to frequently express DDC. The range of DDC values in the colorectal cell lines was similar to that present in cell lines derived from small cell carcinoma of the lung (36), a typical NE tumor. NE cells contain dense core granules, characteristic of endocrine secretion, present in every cell. While rare osmiophilic granules have been described in colorectal carcinomas by electron microscopy and silver staining (37–39).

We investigated production and secretion of 3 membrane associated glycoproteins. CEA, which has been extensively investigated, is present on normal and malignant colonic tissues as well as other cancers (22). It is both a tumor tissue and a serum marker (22, 23). It is actively produced and secreted by colon carcinoma lines, although levels vary widely (24). Controversy exists as to whether high levels are associated with well or poorly differentiated lines (24, 25). CA19-9, originally identified from a colon carcinoma line, is a sialylated lacto-N-
same tumor, the illustrations are of low power and cannot be critically evaluated. NCI-H716 also grew after i.p. inoculation in athymic mice, although unlike NCI-H498, it formed solid tumor masses without ascites. Because these granules, characteristic of NE cells, were not present in other DDC positive lines, the latter only express part of the NE program.

Cytogenetic studies indicated that 1 cell line was pseudodiploid while the other 10 lines examined were aneuploid with modal chromosome numbers ranging from 47 to 102. An unexpected finding of great potential interest was the presence of DM chromosomes in 9 of 11 lines. In 6 of the 9 positive lines they occurred in high frequency in the majority of metaphase spreads. In addition, 3 lines, all from previously treated patients, contained HSRs. In one cell line some of the DMs were larger than usual, as described by Bullerdiek et al. (411), and are termed chromatic blocks. DMs and HSRs are characteristics of cells having amplified gene sequences, especially protooncogenes and genes associated with drug resistance (42–47). Previous reports have indicated that the finding of DMs and HSRs in colon carcinoma lines is infrequent (48). However, COLO 320, a colon line reputed to have neuroendocrine properties, had many DMs which were later replaced by a HSR (40). The \( \text{myc} \) gene family, are among the protooncogenes frequently associated with cytogenetic evidence of gene amplification (46, 47), and \( \text{c-myc} \) amplification is present in COLO 320 (49). While all of our lines expressed \( \text{c-myc} \) mRNA, only one line, NCI-H716, has amplified gene sequences. This line, and the two others with HSRs were derived from previously treated patients. Identification of the amplified gene sequences in our lines may provide important clues to the pathogenesis of colorectal cancer. The in vitro chemosensitivity patterns of our cell lines to cytotoxic drugs are reported elsewhere (50).

Our well characterized lines constitute a panel consisting of multiple subtypes and include two possibly unique cell lines. They are useful systems for the study of glandular and NE differentiation, antigen expression, and mucin secretion. Identification of the gene (or genes) apparently amplified in many of the lines may provide clues to the pathogenesis of colorectal carcinoma.

REFERENCES


Characteristics of Cell Lines Established from Human Colorectal Carcinoma

Jae-Gahb Park, Herbert K. Oie, Paul H. Sugarbaker, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/24_Part_1/6710

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.