Cell Culture of Human Colon Adenomas and Carcinomas

James K. V. Willson, Gerard N. Bittner, Terry D. Oberley, Lorraine F. Meisner, and James L. Weese

ABSTRACT

Cell lines were established from colon adenomas, including tubular and villous polyps, primary adenocarcinomas, and metastases arising in patients with colon adenocarcinomas. The protocol for cultivating these diverse tissues includes primary cultivation of tissue explants on a type I collagen gel followed by nonenzymatic subculture of the epithelial outgrowth. All early passages were accomplished using low subculture ratios. Cultured cells elaborate morphological structures which are similar to features present in the tissues from which they were cultivated. Specifically, all structural features of colon epithelial cells were identified, including junction formation, prominent microvilli, and mucin secretion, in several cell lines. Five cell lines cultured from colonic neoplasms at different stages of cancer progression were selected for detailed characterization. Cells grown from two tubular polyps had normal human karyotypes. Cells from a villous polyp and all adenocarcinomas were aneuploid with stable marker chromosomes. The established cell lines exhibit distinct phenotypes based on growth characteristics in vitro and in athymic mice; and it is suggested that these cell lines represent useful models for studying the evolution of colon cancer from a benign to an aggressive cell type.

INTRODUCTION

Our current understanding of the natural history of colon carcinoma is based primarily upon clinical observations which suggest that this disease arises within adenomatous polyps rather than the normal bowel mucosa (2-6). Not all adenomatous polyps, however, develop into cancers. Colon carcinomas are 10-fold more frequent in villous polyps than in tubular polyps and hyperplastic lesions are rarely, if ever, the site of carcinomas (5). Furthermore, once carcinomas have developed, the metastatic potential of an individual colon carcinoma varies considerably from patient to patient. Evidence of this variability includes the fact that approximately one-half of all patients with the diagnosis of colon carcinoma are cured by surgical resection alone (7, 8). Clinical studies have indicated that colon carcinomas do not metastasize until invasion of the bowel mucosa has occurred and the degree of bowel wall invasion predicts the likelihood that metastatic disease will develop (9, 10). These clinical observations imply that the transition from a benign polyp to a metastatic bowel cancer involves sequential steps during tumor progression.

Recently, considerable attention has been focused on identification of the molecular and biological alterations associated with this evolution from a benign to a metastatic cell. Events which have been suggested by studies in laboratory systems as significant in this evolution include increased transcription of certain oncogenes necessary for cell proliferation (11-13); loss of requirement for specific growth factors leading to autologous growth stimulation (14-16); and deficiencies in normal growth control mechanisms mediated through cell-to-cell contact (11). A model system which includes cells representative of the adenomatous polyp, the minimally invasive colon adenocarcinoma still curable by resection, and the metastatic phenotype would provide a very useful system to evaluate the role of these events in the progression of colon carcinoma.

To develop such a model we have modified recently described techniques which had been used to establish cell lines from the majority of colon carcinomas in which metastases had occurred. The previously reported techniques were not useful for cultivation of the nonaggressive surgically curable cancer phenotype or for in vitro propagation of adenomatous polyps (17). In this report we describe culture methods which have been used to derive cell lines from adenomatous polyps, early stage carcinomas, and metastases. A detailed characterization of cell lines cultured from two tubular polyps, a villous polyp, a well-differentiated Dukes' Stage B1 adenocarcinoma, and a hepatic metastasis occurring in a patient with a previous Dukes' Stage C colon carcinoma is presented.

MATERIALS AND METHODS

Clinical Specimens. Biopsies of polyps, primary colorectal carcinomas, and metastases were obtained at the time of exploratory surgery either for colectomy or surgical placement of a hepatic artery infusion catheter or via a colonoscopic-directed biopsy. A representative portion of the surgical specimen was taken aseptically and transported from the surgical suite in cold (4°C) tissue culture medium for immediate processing. A representative portion of the tissue was fixed and stained with hematoxylin and eosin.

Cell Culture Materials. The basic culture medium consisted of MEM* with Earle's salts supplemented with 0.1 mM nonessential amino acids, 4 mM L-glutamine (all from Grand Island Biological Co., Grand Island, NY), and gentamicin (50 μg/ml; Schering, Kalamazoo, MI), MEM-2+ was MEM supplemented with 2% FBS (Sterile Systems, Logan, UT), insulin (10 μg/ml), transferrin (2 μg/ml), hydrocortisone (1 μg/ml), and triiodothyrine (10^{-8} M) (all from Sigma Chemical Co., St. Louis, MO), and sodium selenite (5 × 10^{-8} M) (DIFCO, Detroit, MI).

Collagen substrate used for primary cell cultures from explants and for maintenance culture was prepared in 35-mm dishes (6-well culture dishes; Costar, Cambridge, MA). Collagen substrate preparation followed previously described procedures (18, 19). In brief, 1 g of dried tendons from rat tails was dissolved in 300 ml 0.1% acetic acid distilled water for 48 h at 5°C. The solution was centrifuged at 14,000 x g; 300 ml of the supernatant was used. The minced tissue was collected and used as a stock collagen solution which contained 1.2 ± 0.25 mg protein/ml. A 1.0-ml aliquot of stock collagen solution is placed in a 35-mm dish and exposed to the vapors of NELOH for 10 min. The plate is then maintained in MEM and 8% FBS and then exposed to MEM-2+ for 2 to 24 h prior to initiation of explant culture.

Conditions for anchorage-independent cloning assays included MEM supplemented with 15% FBS, insulin (5 μg/ml), 100 μM ascorbic acid, 2 mM glutamine, 10 μM nonessential amino acids, and 50 μg/ml gentamicin.

Received 10/31/86; revised 2/4/87; accepted 2/12/87.

The content of publication of this article was 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.

This investigation was supported by the Veterans Administration Medical Research Service and was presented in part at the 76th Meeting of the American Association for Cancer Research at Houston, TX, 1985 (1).

2 To whom requests for reprints should be addressed, at Division of Medical Oncology, B-5055, William S. Middleton Veterans Hospital, 2300 Overlook Terrace, Madison, WI 53705.

3 Present address: Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111.

4 The abbreviations used are: MEM, minimum essential medium; MEM-2+, minimum essential medium supplemented with 2% fetal bovine serum and sodium selenite; FBS, fetal bovine serum; HBSS, Hank's balanced salt solution.

2704
biate, 2.0 mM pyruvate, 2 mM L-glutamine, 0.2 mM nonessential amino acids, and 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid all gelled with agarose (low electroendosmosis; Seachem, FMC, Rockland, ME) to a final concentration of 0.3% for the bottom layer and 0.15% for the plating layer.

**Primary Explant Culture and Subculture Procedures.** Fresh colon neoplastic tissues were minced with apposed scalpels into 0.5-mm pieces. Individual pieces of tissue were placed 6 to 8 per dish on a previously formed collagen substrate. During the attachment process only medium sufficient to wet the substrate surface was used. After 10 to 12 h an additional 2 ml of MEM-2+ was added to each dish.

Explants were incubated in a humidified atmosphere of 5% CO₂ in air at 37°C. Cultures were observed daily, and medium was changed three times a week. Epithelial outgrowth at the periphery of the explant was evident within 24 to 48 h of attachment. This outgrowth increased over time but never became confluent.

Subculture was not attempted in most cases until 2 to 3 weeks. After 10 to 21 days in culture the expansion of epithelial outgrowths would decrease and often multilayered patterns of growth would form. When the rate of peripheral outgrowth was noted to decrease, subculture was attempted. To selectively detach epithelial cells from outgrowths the primary cultures were exposed to multiple 3-min exchanges of 2 ml of HBSS (GIBCO) containing 0.04% EDTA at 37°C. Exchanges of HBSS without Ca²⁺ or Mg²⁺ and EDTA were continued until the epithelial cells forming the outgrowths from primary explants rounded up. Usually three to four exchanges were sufficient. Single cells and small cell clusters (5 to 10 cell aggregates) were dislodged from the collagen gel following gentle pipeting. The cells were washed in Ca²⁺ containing MEM and 2% dialyzed fetal bovine serum for 5 min at 200 μg/L. Cells were resuspended in MEM-2+ and then seeded onto collagen gels in 35-mm wells. The initial passage ratios were not greater than 1:2. These subculture methods evolved during the period in which cell lines derived from tubular polyps have been passaged at one to eight split ratios each week. Cells were expanded on collagen and after the initial subculture these cell lines were expanded in T-25 flasks. The continuous cell lines were expanded on collagen and after the initial subculture these cell lines have been passaged at one to eight split ratios each week. Cells derived from tubular polyps have been passaged at one to two split ratios. Optimal seeding efficiency of the cells derived from adenomatous polyps was accomplished when the medium used was 2 parts conditioned MEM-2+ and one part fresh MEM-2+. Conditioned MEM-2+ was obtained by exposing MEM-2+ for 48 h to either the primary explant culture or subcultures of the cell line being passaged.

**Morphology.** Electron micrographs were taken with a Phillips transmission electron microscope from thin-sectioned material embedded as follows. Epithelial cell cultures were refed with medium 24 h prior to fixation with glutaraldehyde and 0.4% OsO₄ and carried through graded ethanol solutions for dehydration prior to propylene oxide exchange and Epon 812 infiltration; 80-nm sections were stained with lead citrate and uranyl acetate.

**Tumorigenicity.** Six-week-old female athymic mice (Sprague-Dawley, Madison, WI) were housed five mice/cage in an isolator and fed autoclaved food ad libitum. Tumor xenografts were grown by inoculating animals s.c. with 5 × 10⁶ cells/0.2 ml in the scapular area. Mice were observed for development of tumors for 3 months. Representative xenografts were fixed and sectioned for histological staining with hematoxylin and eosin.

**Karyotype Determination.** Cells were refed with fresh growth medium 24 h prior to the addition of Colcemid (0.1 μg/ml; Grand Island Biological Co.). One to 4 h later, cells were disaggregated, incubated in 0.075 M KCl for 20 min at 37°C, and fixed with 25% glacial acetic acid in anhydrous methanol. Trypsin-Giemsa banding was performed, and multiple karyotypes determined.

**Mycoplasma Detection.** Cells cultured in antibiotic-free media were submitted to the American Type Culture Collection Service, Rockville, MD, and to the Wisconsin State Laboratory of Hygiene, Madison, WI, to monitor for mycoplasma contaminations. Both aerobic and anaerobic cultures for mycoplasma were followed for 1 month. The cells are screened routinely by Hoechst Stain (Flow Laboratories, McLean, VA). Cell lines discussed in this report have been consistently free of mycoplasma contamination.

**RESULTS**

Continuous cell lines have been derived from diverse human colon neoplastic tissues including a villous polypl, a well-differentiated Duke's Stage B1 adenocarcinoma of the sigmoid colon, and a hepatic metastasis which occurred in a patient with adenocarcinoma of the colon (Fig. 1). All cell lines were established from epithelial outgrowth from tissue explants cultivated on a type I collagen gel substrate. Using similar methods, finite cell lines were routinely established from adenomatous polyps and in two cases cells cultured from two separate tubular polyps have been expanded and are currently maintained in T-25 flasks. In the next section a detailed discussion of the methods used to establish these cell lines from human colonic neoplastic tissues is presented.

**Primary Explant Culture and Subculture on Type I Collagen.** We had previously developed highly successful techniques for the cultivation of cell lines from human colon cancer which had demonstrated metastatic behavior in the clinic; however, using these techniques we were not able to derive lines from less aggressive colonic neoplasms (17). Because others had used various substrates derived from extracellular components to facilitate the primary culture of normal (19, 20) as well as benign neoplastic epithelial tissues (21), we decided to use a similar approach for cultivation of colonic polyps and cancers with low metastatic potential. In preliminary experiments reported elsewhere (22), we found that tissue explants would attach and produce epithelial outgrowth if cultivated on a type I collagen gel substrate in a medium supplemented with insulin, transferrin, sodium selenite, hydrocortisone, and triiodothyronine. Explant attachment and epithelial outgrowth on the type I collagen gel were superior to that observed with plastic or air-dried collagen substrates (22). High, 10%, concentrations of FBS in the growth medium were associated with fibroblast contamination; however, medium supplemented with 2% FBS resulted in minimal fibroblast contamination (22).

Using this primary explant method, all adenomatous polyps and 69% of the carcinomas attached and produced epithelial outgrowth which could be subcultured, Table 1. In most cases it was possible to subculture this epithelial outgrowth after 10–21 days of primary culture. A key to successful subculture was use of a nonenzymatic method for dispersing the attached epithelial cell outgrowth, accomplished by three to four three-min exposures of the primary culture to HBSS and 0.04% EDTA at 37°C. Epithelial cells at the periphery of the explant would round up; single cells and small aggregates (5 to 10 cells) would release into the medium with gentle pipeting; and these cells and small aggregates would attach and proliferate when subcultured on type I collagen. Subculture ratios of 1:2 gave the most consistent success. Higher initial subculture ratios were often associated with unsuccessful passage. Subculture was not successful when enzymatic methods with either trypsin or Pronase were used to dissociate epithelial outgrowth.

Cells from tubular, tubulovillous, and villous polyps were cultured as fine cell lines on collagen for multiple passages, Table 1. It was possible to routinely subculture these polyp-derived cells for multiple passages in 35-mm wells, but expansion of the cell number was limited because of low seeding efficiency when cells were passaged. During the course of this study, we found that the seeding efficiency of tubular polyp...
Fig. 1. Light microscopy of tissues used in subsequent in vitro studies. **A**, Light micrograph of epithelium of well-differentiated adenocarcinoma. Cells are arranged in glands with most of the cells showing basilar nuclei. **Arrows**, Mitoses in the glands. **B**, Light microscopy of cells from colon (well-differentiated adenocarcinoma) culture injected into nude mice. The cells attempt to form glands and mitoses (arrows) are evident. **C**, Light microscopy of adenomatous polyp. The cells have basilar nuclei. Mitoses are infrequent. **D**, Light microscopy of metastasis in liver. Malignant tumor cells are identified (arrows), and lymphocytes are seen surrounding tumor cells.

### Table 1 Primary and continuous culture of human colon neoplastic cells

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Number cultured</th>
<th>Number of primary cultures established</th>
<th>Number of cell lines established</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenomas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubular and tubulovillous polyps</td>
<td>8</td>
<td>8</td>
<td>2†</td>
</tr>
<tr>
<td>Villous polyps</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Carcinomas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>23</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Metastases</td>
<td>22</td>
<td>16</td>
<td>7</td>
</tr>
</tbody>
</table>

*In eight of eight tubular or tubulovillous polyps finite cell lines were cultured for two to 18+ passages (median no. of passages, 3). In two cases 310 and 330 expanded cell lines continue to be passaged.

It is important to point out that the VACO 310 and 330 cell lines are not necessarily immortalized; however, the ability to expand their numbers as cell lines will permit molecular and biochemical characterization studies not possible with primary cultures.

A continuous cell line was derived from a villous polyp, VACO 235, without using conditioned medium. This cell line has been maintained for 50+ passages over an 18-month period. The VACO 235 cell line was derived from a superficial biopsy of a villous polyp arising in the rectum of a 50-year-old woman. The biopsy from which the VACO 235 cell line was established contained areas of dysplasia but no carcinoma. A subsequent resection of the rectal polyp revealed a carcinoma at the polyp base.

Continuous cell lines were grown from a broad range of colonic adenocarcinomas cultured initially as explants on collagen gels, Table 1. Continuous cell lines were derived from 13...
colon adenocarcinomas (6 primary and 7 metastases). Explants from 69% (31 of 45) of colon adenocarcinomas gave primary epithelial outgrowth on collagen. While resources did not permit us to attempt to subculture cells from all primary cultures, we established lines from all 13 adenocarcinomas which were subcultured. Conditioned medium was not required for expansion of the cell lines derived from adenocarcinoma.

Five cell lines will be described in detail because they have been derived from cellular phenotypes representative of the clinical progression of colon carcinoma from adenomatous
CELL CULTURE OF HUMAN COLON ADENOMAS AND CARCINOMAS

Fig. 3. Transmission electron microscopy of a villous polyp in culture, VACO 235. A, Adenomatous polyp cells grown on collagen substrate (passage 10). The cells have prominent mucin (M) in their cell cytoplasm. The cell surface shows microvilli with core rootlets. There are numerous mitochondria in the cell cytoplasm. There are junctional complexes seen between cells. Inset, microvilli have a prominent glycocalyx (arrow). There are junctional complexes seen between cells, especially prominent on the apical surface. B, Adenomatous polyp cells grown on collagen substrate after subculture, passage 35. The cells attempt to form glands, and the glands are lined by microvilli which have prominent core rootlets. There are tight junctions seen between cells. On collagen the cells tend to form multilayer structures. The mucin and glycocalyx seen in passage 10 were lost with further subculture.

polyp to metastatic cancer. These cell lines include cells derived from two tubular polyps, VACO 310 and 330; a villous polyp, VACO 235; a well-differentiated Dukes' B1 colon carcinoma, VACO 206; and a hepatic metastasis, VACO 241. The morphology, in vitro and in vivo growth characteristics, and cytogentic of these selected lines are presented in the following sections.

Morphological Characterization of Cell Lines. The cells cultivated on type I collagen gel elaborate morphological features that are similar to those present in the original tissues. To illustrate this point, the ultrastructural morphology of the cultured cells, Figs. 2–5, are shown. Cells cultivated from two tubular polyps, VACO 310, Fig. 2A and VACO 330, Fig. 2B, exhibit features of a well-differentiated epithelial cell including microvilli with core rootlets and junctional complexes which are particularly prominent on the apical surfaces. The polarity of these cultured polyp cells is striking with basal nuclei, apical microvilli, and junctional complexes.

Passage 10 of cells grown from a villous polyp, VACO 235, are shown in Fig. 3A. These cells also exhibit well-differentiated epithelial structures which are maintained with passage as illustrated by the fine morphology of passage 35, Fig. 3B. Note that the prominent mucin and glycocalyx seen in the early passage of VACO 235 were lost during subculture, yet cell polarity and other epithelial structures persist.

The well-differentiated features of the Dukes' B1 adenocarcinoma, Fig. 1A, were maintained when that cell was cultivated on type I collagen. These features of epithelial differentiation include basal nuclei and apical microvilli which are evident in the cultured cells, Fig. 4. The VACO 206 cell has maintained these features after multiple passage on type I collagen.

Metastatic colon cancer cells cultivated from a hepatic metastasis, VACO 241, in contrast to the previously described cells, had undifferentiated features in culture, Fig. 5. While a few microvilli and occasional junctional complexes are seen, the prominent features of these cells are the large bizarre central...
Fig. 4. Transmission electron microscopy of well-differentiated adenocarcinoma cells VACO 206, in vitro. A, Cells grown on a plastic substrate; the cells consist of a monolayer. Arrows, microvilli with core rootlets on cell surface; arrowhead, junctional specializations between cells. Occasional mitochondria and a moderate amount of rough endoplasmic reticulum are seen in the cells. B, Well-differentiated adenocarcinoma cells grown on a collagen substrate; mitoses (M) are observed. The cell surface shows prominent microvilli. Arrows, prominent junctional complexes between cells. The cells form a monolayer. There is a moderate amount of rough endoplasmic reticulum present, and mitochondria are seen. C, Well-differentiated adenocarcinoma cells in vitro grown on a collagen substrate after numerous passages. Arrows, after numerous passages, the cells still retain microvilli with core rootlets. However, now numerous microfilaments (MF) are seen in the cell cytoplasm.
junctional complexes are seen between the cells. Arrowhead, some cells show
weeks.

Methods." triplicate 35-mm wells on a collagen gel and counted daily for 5 days.

Mitoses (M) are seen. There are occasional mitochondria
in vitro, VACO 241. Arrows, a few microvilli on the cell surface. Occasional
intracellular lumens. Mitoses (M) are seen. There are occasional mitochondria

Anchorage-independent growth was assessed using a two-
layer agarose gel culture, Table 2. Only VACO 241, derived
from a hepatic metastasis, grew in agarose with a cloning
efficiency of 2.2%.

The VACO 206 and 241 cells formed xenografts in athymic
mice from a s.c. inocula of 5 × 10⁶ cells. Xenografts formed
from the VACO 206 cells exhibited well-differentiated features
including glandular structures formed by cells having basilar
nuclei, Fig. 1B. Cells derived from adenomas failed to form
xenografts in athymic mice injected with 10⁷ cells and observed
for 10 weeks.

The biological characterization of these lines has identified
three biological phenotypes each of which can be correlated to
a clinical phenotype. Phenotype 1, the polypl-derived cells, were
not tumorigenic and did not exhibit anchorage-independent
growth; phenotype 2, VACO 206, the Dukes' B1 cancer-derived
cell, was tumorigenic but did not exhibit anchorage-independent
growth; and phenotype 3, VACO 241, the metastasis-
derived cell, was both tumorigenic and exhibited anchorage-

Karyotypes. Two tubular polyp-derived cell lines, VACO 310
and 330, had normal human karyotypes, 46, XY. Distinctive
human karyotypes were found for each of the other cell lines.
The villous polyp line, VACO 235, had a very stable chromosome
constitution characterized by a modal number of 47, with
an extra no. 21 and with nine chromosomal rearrangements
consisting of a deletion of the short arm of one chromosome
no. 1, an isochromosome of the long arm of the other no. 1
chromosome, an interstitial deletion of the no. 3 short arm,
both no. 6 chromosomes with a deletion of half of the long
arm, extra material translocated to a no. 7 and a no. 12, an
isodicentric no. 14, and deleted no. 18 (Fig. 6A). The adenocar-
cinoma line, VACO 206, on the other hand, showed a slightly
more variable karyotype with a modal number of 45 chromo-
somes, with loss of an X, 8, and 18, and with an extra no. 3
and minute marker whose origin could not be identified. VACO
206 had four other chromosomal rearrangements, including a
deleted no. 1, a deleted no. 3, an isochromosome of one no. 15,
and extra material translocated to the other no. 15 long arm
(Fig. 6B). Thus, the villous polyp line and the adenocarcinoma
line, VACO 206, both had a deleted no. 1 and a deleted no. 3,
though the deletions were not exactly the same, while the
adenoma had extra copies of the no. 1 long arm and the
adenocarcinoma line had extra copies of no. 3 and no. 15. In
contrast to the extensive rearrangements in these two cell lines,
the line derived from the metastasis, VACO 241, showed min-
imal changes; the modal number was 46 with loss of the Y
chromosome but with an extra marker chromosome consisting
of an isochromosome of the short arm of a no. 7 chromosome
(Fig. 6C). A tetraploid version of this line was observed in 40%
of the metaphases analyzed.

Table 2 Human colon neoplastic cell lines: in vitro characterization

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Clinical stage</th>
<th>Doubling time (h)</th>
<th>Cloning efficiency (%) agarose</th>
<th>Tumorigenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>VACO 330</td>
<td>Tubular polyp</td>
<td>32</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>VACO 235</td>
<td>Villous polyp</td>
<td>34</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>VACO 206</td>
<td>Dukes' B1 carcinoma</td>
<td>41</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>VACO 241</td>
<td>Hepatic metastasis</td>
<td>29</td>
<td>2.2</td>
<td>+</td>
</tr>
</tbody>
</table>

DISCUSSION

In this report we have described methods which were used to
cultivate cell lines from benign and nonmetastatic colonic neo-
plasms. It is our opinion that the success of these methods was
determined in large part by the use of culture conditions which
preserved cell-to-cell interactions as well as permitted the
expression of characteristic growth behavior by the cultured
cells. These conditions were accomplished by initiating primary
cultures from explants on a type 1 collagen substrate. These
are not new observations. The importance of preserving cell-to-
cell associations for the successful cell culture of colonic epi-
Fig. 6. Modal karyotypes: A, VACO 235, 47,XX, +21,del(1) (p32), i(1q), del(3)(p13::p21),del(6)(q21), del(6)(q21), 7q+, 12q+, dic(14)(pter->q24::q24->pter), del(18)(q21), B, VACO 206, 45, X, 1p-, +3p-, -8, i(15q), 15q+, -18, + minute marker; C, VACO 241, 46, X, +i(7p).
thelial tissues was noted by us in our previous experience with the cultivation of clinically aggressive colon carcinomas (17), as well as by Moyer in the cultivation of normal colon mucosa (23); Friedman (21) in the primary culture of colonic adenomas; and Leibovitz (24) in the establishment of cell lines from colon carcinomas. Many of the well-differentiated features exhibited in the original tissues such as cell polarity and structures which characterize well-differentiated colon epithelial cells were present when the cells were grown on type 1 collagen. Subculture was possible if nondenzy nic dissociation and low split ratios were used during the early passages. Importantly, the well- differentiated features of these neoplastic epithelial cells were preserved when subcultured on type 1 collagen.

The successful cultivation of benign colonic epithelial tissues has been accomplished by others. Moyer (23) has successfully maintained normal colonic epithelium in culture, Paraskeva et al. (25) has reported on the establishment of cell lines from polyps obtained from patients with familial polyposis, and Friedman (21) has described a primary culture system for adenomatous polyps on a collagen substrate. Our culture methods have extended this experience with the establishment of a villous polyp cell line, VACO 235, and methods to routinely subculture tubular and tubulovillous polyps, and in two cases, VACO 310 and 330, expand in culture cells from tubular polyps. It is intriguing to speculate that the VACO 235 polyp cell had progressed further than the other polyp-derived cells which we have cultured. The presence of an invasive carcinoma at the base of the villous polyp from which an earlier biopsy had been obtained and used to establish the VACO 235 cell line supports this possibility. The occurrence of such progression in VACO 235 cells may explain why a continuous cell line could be readily cultivated without conditioned medium from this polyp.

The cell lines developed in this study were tested for ability to form xenografts in athymic mice, tumorigenicity, and for clonogenic growth in agarose, anchorage-independent growth, both laboratory characteristics associated with the cancer phenotype. Three phenotypes were identified. Polyp-derived cells were not tumorigenic and did not exhibit anchorage-independent growth; a Dukes' B1 adenocarcinoma (invasion into but not through the serosa of the bowel wall) which was tumorigenic but did not exhibit anchorage-independent growth; and an hepatic metastasis which was both tumorigenic and exhibited anchorage-independent growth. Thus the acquisition of tumorigenic potential and growth in anchorage-independent conditions are associated in this cell line model system with the progression from an adenomatous polyp to an invasive and then metastatic bowel cancer. These cell lines may be useful in the identification of the molecular events which led to these phenotypic changes and by association to a more aggressive phenotype.

Comparison of the karyotypes of the three cell lines shows that only the adenomas retained the second sex chromosome, the X and Y, respectively, have been lost from the adenocarcinoma and the metastasis. What is most curious is the fact that the villous polyp contained nine rearranged marker chromosomes seen. For example, many investigators consider loss of one copy of the no. 1 short arm to be related to progression (26), while an extra copy of the no. 1 long arm, whether by translocation or isochromosome forma tion, is thought to occur at a relatively late stage of malignant development, and is a change thought to contribute to the progression of all types of cancer (27). A previous report of the karyotypic findings in a villous adenoma noted that such a duplication of the long arm of chromosome no. 1 was present in three of 30 cells examined, and the authors postulated that if the 1q+ had been present in more cells, the tumor would have progressed to invasive neoplasia (28). This speculation on the significance of the duplication of the number 1 long arm is supported further by the fact that the villous polyp from which VACO 235 was derived had progressed to a carcinoma at a site distant from that cultured. A more detailed discussion of the chromosome changes in this villous adenoma and their possible significance appears elsewhere. 5

Although the adenoma shows the duplication of the no. 1 chromosome associated with advanced malignancy, the adenocarcinoma and the liver metastasis do not. Other changes seen in these cell lines which have been previously reported in large bowel tumors include trisomy 21, one of the most frequent trisomies in such tumors (29), which was observed in the adenoma. Loss of no. 18, another primary change associated with cancer of the large bowel (30, 31), was observed in the adenocarcinoma. Trisomy of the chromosome no. 7 is among the most frequent trisomies in bowel tumors (17, 22), yet finding an isochromosome of just the short arm as the only chromosome aberration in the metastasis is most unexpected, especially in view of the more aberrant karyotypes seen in the other less aggressive cell lines. Thus, although all of the chromosomal changes described here have been observed in one form or another in bowel tumors, most studies suggest more rather than less rearrangement and aneuploidy in the course of cancer progression. Indeed, according to recent reports, aneuploidy is a poor prognostic factor in patients with bowel cancer (31), yet despite the aggressive nature of VACO 241, these cells would not have been judged aneuploid in such studies.

What is the significance of the unexpected chromosomal findings in the three cell lines described here? Although numerical and structural chromosome changes usually increase with time in a malignant cell line, suggesting that genetic instability may be adaptive in terms of invading new microenvironments, the specific chromosomal segments that are duplicated in any given neoplasm may well have been selected on the basis of the genetic constitution of the host as well as the requirements of the localized microenvironment (32). Thus, when a truly adaptive chromosome pattern emerges, further instability and chromosomal rearrangement could prove counterproductive, and this is why one can see an undifferentiated metastatic cell line such as 241 with fewer chromosome changes than is seen in the less aggressive lines. Despite many chromosomal changes in the villous polyp and the adenocarcinoma, they both remained differentiated, indicating that none of these changes had conferred the adaptive advantage of the visible as well as the submicroscopic changes that must have taken place in line 241. The fact that the villous polyp had more chromosome changes than the adenocarcinoma, and was quite stable in terms of its chromosome pattern, suggests that these many changes alone were not enough to confer malignancy.

In summary, we have described cell lines derived from tubular and villous polyps, an early carcinoma and a hepatic metastasis. These cells extend our previously reported cell line bank to include cells derived from a precancerous tissue and a nonaggressive colonic cancer. The cell lines reported here represent a model which may be used to probe the genetic and biological

---

5 Meisner, L. F., and Wilson, J. K. V. Chromosome changes in a villous adenoma, manuscript in preparation.
difference between transformed colonic epithelium which is localized and curable with surgery and the cancer which cannot be cured by resection. It is hoped that a better understanding of this process will lead to the design of strategies to more successfully attack the aggressive colon cancer phenotypes.

REFERENCES


Cell Culture of Human Colon Adenomas and Carcinomas


Updated version  Access the most recent version of this article at:  http://cancerres.aacrjournals.org/content/47/10/2704

- **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, use this link http://cancerres.aacrjournals.org/content/47/10/2704. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.