Variants of a Human Colon Adenocarcinoma Cell Line Which Differ in Morphology and Carcinoembryonic Antigen Production


INTRODUCTION

A major problem encountered in research with human cancer cells cultured in vitro is the inability to establish lines of normal epithelial cells; hence, comparative studies of normal and neoplastic cells are difficult to perform. This problem may be circumvented by studies with cloned variants of a cancer cell that differ in 1 or more properties. In this manner a particular cellular characteristic, either lacking or enhanced in a cloned strain, may be investigated and correlated to specific properties such as tumorigenicity. Recently, a continuous line of human colon adenocarcinoma cells cultured in vitro is the inability to establish lines of normal epithelial cells; hence, comparative studies of normal and neoplastic cells are difficult to perform. This problem may be circumvented by studies with cloned variants of a cancer cell that differ in 1 or more properties. In this manner a particular cellular characteristic, either lacking or enhanced in a cloned strain, may be investigated and correlated to specific properties such as tumorigenicity. Recently, a continuous line of human colon adenocarcinoma cells (HCT-8) was isolated and cloned. The variant and parent cell line were characterized with respect to morphology, growth characteristics, karyotype, production of CEA, and ability to form tumors in nude mice. The variant cells differed from the parent cells in morphology, marker chromosomes, and ability to form colonies in soft agar and produced more carcinoembryonic antigen. The two cell strains were equally oncogenic in nude mice, although HCT-8R cells produced poorly differentiated tumors while HCT-8 cells produced tumors with both differentiated and poorly differentiated areas. Thus, in nude mice, no correlation was observed between carcinoembryonic antigen production by cells and their oncogenicity.

MATERIALS AND METHODS

Cell Cultures. The origin and characterization of HCT-8 cells has been described previously (21). A strain of cells was derived from these cells by aspirating a focus of morphologically distinct cells from a monolayer of HCT-8 cells with a capillary pipet. The aspirated cells were subsequently cloned, and a selected clone was grown into monolayers which were passaged. These cells, designated HCT-8R, were used between passages 5 and 40 in the present study. For establishment of cultures of cells from tumors grown in nude mice, the tumors were excised and processed by the method used to establish the HCT-8 cells (21). An established cell line of human embryonic intestine cells used as controls in CEA studies was obtained from Grand Island Biological Co. (Grand Island, N. Y.). All cultured cells were grown and passaged as previously described (21). The cultures were periodically tested for Mycoplasma (9) and were consistently found to be free of these organisms.

Morphology. Tumor cell and colony morphology were studied with the use of light, phase-contrast, and transmission electron microscopy as described previously (21).

Cell Growth. Cell population-doubling time, plating efficiency, and mitotic index were determined as described previously (21). For assessment of colony formation in soft agar, a 0.5% agar (Bacto-Agar; Difco Laboratories, Inc., Detroit, Mich.) was added to form a base layer in 60-mm Petri dishes. A 2nd layer of 1.5 ml containing a suspension of about 250 cells in 0.3% agar was added on top of the base layer. After 7 to 14 days of incubation at 37°, the cultures were stained with a 0.005% neutral red solution. The dye diffused through the agar, and cell viability, colony formation, and size were assessed by microscopic and macroscopic examination.

Detection of H Blood Group Antigen. HCT-8 cells were derived from a carcinoma of a patient of the O blood type (16). The specific RBC adherence test was used to detect the presence of H antigen (4).

Cytogenic Analysis. For chromosome analysis, Colcemid was added to the culture bottles at a final concentration of 0.1 μg/ml 2 hr before harvesting. The cells were treated with 0.075 M KCl for 30 min and fixed in methanol:acetic acid (3:1, v/v). The slides were flame dried and stained with 0.5% Atebrin according to the method of Lin et al. (11). The slides were scanned, and metaphases were photographed with a Zeiss fluorescent microscope under darkfield illumination. The cell cultures under study were coded, and the karyotypic analyses were recorded before the code
was broken. Between 5 and 10 karyotypes were made from each of the cultures, and an additional 5 to 10 photographs of metaphases were analyzed for marker chromosomes.

**Tumorigenicity in Nude Mice.** Cultures of the various cell strains were trypsinized, washed, and resuspended in TBS. The cells were enumerated, and their viability was assessed by trypan blue dye exclusion. Homozygous (nu/nu) nude RNC mice were obtained from Dr. P. Miniats of Guelph University (Guelph, Ontario, Canada). Groups of 5 nude mice, 5 to 8 weeks old, were given injections s.c. on the back of various doses of cells ranging from 10^2 to 10^7 cells in 0.1 ml of TBS. The mice were maintained in separate quarters under conventional conditions and observed daily for 2 months for tumor formation. Select tumors were excised and processed for either cell culturing or histological examination. For histology, portions of tumors were fixed in 10% buffered formalin, processed, and embedded routinely. Five-μm sections were stained by standard hematoxylin-eosin and periodic acid-Schiff techniques. Approximately 40% of the animals bearing tumors were autopsied for metastasis.

**CEA Determination.** Production of CEA by the various cell strains was determined by assaying the supernatants from 8-day-old cultures. The culture medium was collected, centrifuged at 75 × g for 10 min to remove cell debris, and stored at −20°C until tested. Under code, the CEA content of supernatants was measured by the Z-gel method of LoGerfo et al. (12) with reagents obtained from Hoffmann-La Roche, Inc. (Nutley, N. J.). Cell surface CEA was detected by indirect immunofluorescence; the source and specificity of the reagents as well as details of the methodology are described elsewhere (16).

Cell surface CEA was quantitated by assaying the direct binding of 125I-labeled anti-CEA IgG. For this test, globulins were precipitated from NGS and goat anti-CEA serum with ammonium sulfate as described (3). The precipitates were resuspended and dialyzed against 0.01 M sodium phosphate buffer, pH 7.6. The IgG was further purified by DEAE-cellulose 52 column chromatography. The protein concentrations of the IgG-containing fractions were determined by the method of Lowry et al. (13). The samples were diluted to 1 mg IgG per ml, and 50 μg of NGS IgG or anti-CEA IgG were iodinated by the chloramine-T method (14). The 125I was obtained from New England Nuclear, Ltd. (Lachine, Quebec, Canada). Free 125I was separated from 125I-labeled IgG by Sephadex G-25 column chromatography, and the 125I-labeled IgG was used within 24 hr of preparation. In an assay for IgG binding, cells from 3-day-old cultures were monodispersed with trypsin, washed with TBS, and resuspended in TBS at a concentration of 5 × 10^6 cells/ml. Cells were aliquoted into siliconized 12- × 75-mm glass test tubes at 1 ml/tube. The cells were pelleted by centrifugation at 75 × g for 5 min, and the supernatants were decanted. To the cells were added 50 μl of either 125I-labeled IgG from NGS or 125I-labeled anti-CEA IgG. Antibody was allowed to react for 45 min at room temperature after which the cells were washed 5 times with TBS, transferred to a new test tube, and counted in an automatic gamma counter. All assays were performed in triplicate and specific binding was calculated by subtracting the average cpm of 125I-labeled NGS IgG from the average cpm of 125I-labeled anti-CEA IgG.

**RESULTS**

**Derivation of an HCT-8R Substrain of Cells.** Foci of morphologically altered cells were observed in monolayers of HCT-8 cells. A focus of cells was aspirated from the monolayers, grown in culture, and subsequently cloned. The cloned variant was designated HCT-8R.

Morphologically, HCT-8R cells appeared pleomorphic and colonies contained fusiform, epithelial-like, and occasional giant cells. This morphology was distinctly different from colonies of HCT-8, which contained tightly packed epithelial cells (Fig. 1, a and b). Ultrastructurally, HCT-8R cells differed from HCT-8 cells in that they lacked junctional complexes and their microvilli appeared irregular in length, shape, and spacing as opposed to the regular brush-border appearance of microvilli of HCT-8 cells (21).

The growth characteristics of HCT-8R cells are summarized in Table 1. The doubling time, mitotic index, and colony-forming efficiency were similar to those reported for the parent cells, HCT-8 (21). As with HCT-8 cells, the doubling time of HCT-8R cells decreased at higher in vitro passage levels. When equal numbers of HCT-8 and HCT-8R cells were plated in soft agar and examined after 10 days, 75% of the HCT-8 cells plated formed colonies while the HCT-8R cells formed no colonies. Neutral red uptake suggested that both cell types were viable in soft agar.

In the specific RBC adherence test, fixed preparations of both cell lines were reacted with Ulex lectin extract and tested for agglutination of O and A RBC’s. Both cultures demonstrated specific adherence of RBC’s from O blood type but not A blood type individuals; thus, both HCT-8 cells and HCT-8R cells possessed the H blood group antigens.

**Oncogenicity of HCT-8 and HCT-8R In Nude mice.** Within 10 days after injection of the cells into nude mice (15), tumors were visible on mice receiving 10^7 and 10^8 cells of both cell strains (Table 2). Tumors grew locally and metastasis was never observed. Animals receiving lower tumor cell inocula were observed for a period of 2 months and no tumors appeared. Histological examination of tumors formed by HCT-8 cells showed areas of well-differentiated and areas of poorly differentiated adenocarcinoma (Fig. 2a). Both well and poorly differentiated areas of the tumors produced mucin. HCT-8R tumors appeared poorly differentiated (Fig. 2b) but also produced mucin. The morphological characteristics of tumors formed by both cell types were consistent with those found in colon adenocarcinoma.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Population-doubling time, mitotic index, and colony-forming efficiency of HCT-8R cells</strong></td>
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<tr>
<td><strong>Cell strain</strong></td>
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<tr>
<td>HCT-8R</td>
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An HCT-8 tumor and an HCT-8R tumor were excised and established in culture, and the cell strains were designated HCT-8 Nu 1 and HCT-8R Nu 1, respectively. The HCT-8R Nu 1 cells maintained the HCT-8R morphology, but the cells were smaller (Fig. 1f); measurements of trypsinized cells indicated that the majority of HCT-8R cells had diameters of 3.5 to 4 μm. HCT-8R Nu 1 cells presented with a very homogeneous cell size of 2.5 μm in diameter. The HCT-8 Nu 1 cells also maintained the morphology of the parent HCT-8 cells (Fig. 1e). The HCT-8 Nu 1 and the HCT-8R Nu 1 cells formed tumors in nude mice at lower cell concentrations than did the parent cells (Table 2).

Selected tumors formed by HCT-8 Nu 1 or HCT-8R Nu 1 were established in culture, and the cell strains were designated HCT-8 Nu 2 and HCT-8R Nu 2. The HCT-8R Nu 2 cells maintained the morphology and the small size of HCT-8R Nu 1 cells (Fig. 1f). The HCT-8 Nu 2 did not maintain the morphology of the HCT-8 cells; instead of tightly packed epithelial cells, these cells were more fibroblast-like and did not grow in tight colonies (Fig. 1e).

Cyto genetic Analysis. The model number of chromosomes was fairly sharp at 48 for all strains. All cultures from the cytogenetic marker designated 7q+ which was found in blast-like and did not grow in tight colonies (Fig. 1e).

Tightly packed epithelial cells, these cells were more fibroblast-like and did not grow in tight colonies (Fig. 1e).

Table 2: Formation of tumors in nude mice by injected human colon carcinoma cells.

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>10^3 cells</th>
<th>10^4 cells</th>
<th>10^5 cells</th>
<th>10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT-8</td>
<td>5/5</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>HCT-8R</td>
<td>5/5</td>
<td>4/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>HCT-8 Nu 1</td>
<td>ND*</td>
<td>5/5</td>
<td>5/5</td>
<td>2/5</td>
</tr>
<tr>
<td>HCT-8R Nu 1</td>
<td>ND</td>
<td>5/5</td>
<td>4/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* Viable cells injected s.c. on the back of nu/nu mice in 0.1 ml of TBS.
* ND, not done.

Table 3: CEA production by various cell strains derived from a human colon carcinoma.

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>Immunofluorescence % of cells staining*</th>
<th>Binding of [125I]-labeled anti-CEA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>HEI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT-8</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>HCT-8 Nu 1</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>HCT-8 Nu 2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>HCT-8R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT-8R Nu 1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>HCT-8R Nu 2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>HCT-8R Nu 1</td>
<td>100</td>
<td>0</td>
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</table>

* Percentage of cells staining according to degree of intensity of fluorescence; ++, bright; +, moderate intensity; -, no fluorescence.
* CEA concentration in culture medium determined by radioimmunoassay (12).
* cpm of [125I]-labeled anti-CEA IgG specifically bound to 5 x 10^6 cells; results of a representative experiment shown.
* Average ratio of binding ± range derived from 3 experiments. The ratio was derived by dividing cpm for test cell by cpm bound to human embryonic intestinal cells.
* ND, not done.

DISCUSSION

HCT-8, a cell line derived from a human adenocarcinoma...
of the colon, appears to be a heterogeneous cell population. We were able to isolate a variant from cultures of HCT-8 that differed from the parent cells in morphology, marker chromosomes, CEA production, and ability to grow in soft agar. The variant cells, designated HCT-8R, grew as distinct pleomorphic cells, had a 7q+ marker chromosome, produced more CEA than did the parent cells, and did not form colonies in soft agar. The parent HCT-8 cells grew as tightly packed colonies of epithelial cells, had predominantly a 4q+ marker chromosome, and formed colonies in soft agar. The relative production of CEA and the marker chromosomes of the variants were maintained on passing the tumor cells through nude mice, suggesting that the characteristics were stable. Since both strains of cells expressed the H blood group antigen and sex chromosomes of the man from which the cancer was obtained, it is unlikely that the heterogeneity is a result of contamination of the original HCT-8 cells with another cell. Whether this apparent heterogeneity of HCT-8 was present in the original carcinoma or arose after establishing the cells in culture is not known.

Structural alterations of cell surface microvilli were found to accompany malignant changes in studies comparing normal and malignant colon cells (10) and duct cells of dysplastic, benign, and malignant breast tissues (19). We found that HCT-8 cells had uniform microvilli over their free surface (21) and produced predominantly well-differentiated tumors in nude mice. In contrast, HCT-8R cells demonstrated great variability in length, shape, and spacing of villar projections, and these cells produced poorly differentiated tumors in nude mice. Of interest was the finding of poorly differentiated areas found in the tumors induced by HCT-8 cells, suggesting that HCT-8 cells represented a mixture of cells with different phenotypes with respect to the morphology of the cancers produced. HCT-8R cells appear to represent a clonal derivative of the more dedifferentiated phenotype. The finding of variability in the presence and quantity of Regan isoenzyme in various cells of the HCT-8 population by Singer et al. (18) supports the heterogeneity of this cell line. Whether or not this heterogeneity was present in the original tumor specimen or arose during in vitro cultivation is not known.

We found an apparent alteration of tumor cells when grown in nude mice. In the case of both HCT-8 and HCT-8R cultures, the cells showed a significant increase in oncogenicity with the 2nd nude mouse transplantation. Furthermore, on the passage of HCT-8 Nu 1 cells through nude mice, a line of cells (HCT-8 Nu 2) was established that differed morphologically from the parent cell line. HCT-8R, on the other hand, showed neither morphological or cytogenetic changes following passage through nude mice although they became more oncogenic. The ability of cells to change their morphology and biochemical properties after passage through the nude mouse was recently demonstrated for malignant melanoma by Aubert et al. (1). They noted both morphological changes and alterations in the content of 5-S-cysteinylidopa. Similar to our findings, their cells maintained the karyotype of human cells and each strain presented with the specific marker of its corresponding permanent cell line (1).

The association between elevated CEA production with malignant transformation makes this antigen a potential marker for evaluating differences in oncogenicity in tumor cell variants; therefore, HCT-8 and HCT-8R, as well as the nude mouse-passaged cells, were examined for membrane-associated and secreted CEA. In all cases a direct correlation was observed between the amount of cell surface CEA and CEA secreted into the culture supernatant. However, the HCT-8R variant produced significantly more CEA than did the parent HCT-8 cell line. A single passage of either cell strain in nude mice failed to alter the level of CEA synthesis. The ability of human colon carcinoma cells to grow and maintain CEA production in nude mice has also been demonstrated by Carr et al. (2). However, a morphological variant of HCT-8 derived from the 2nd nude mouse passage showed relatively low levels of cell-associated CEA and insignificant levels in the culture supernatant. Thus, it appears possible to select for cells that produce little if any CEA. These results show no correlation between CEA production and oncogenicity for nude mice, suggesting that stimulation of CEA synthesis may not play a critical role in initiation and growth of colon carcinoma.

ACKNOWLEDGMENTS

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REFERENCES

14. McConahey, P. J., and Dixon, F. J. A Method of Trace Iodination of


Fig. 1. Phase-contrast photomicrographs. a, HCT-8; b, HCT-8R; c, HCT-8 Nu 1; d, HCT-8R Nu 1; e, HCT-8 Nu 2; f, HCT-8R Nu 2. × 100.
Fig. 2. a. Histology of HCT-8-induced tumor in the nude mouse. Tumor demonstrates area of good and poor differentiation × 100. b. histology of HCT-8R-induced tumor in the nude mouse × 100.

Fig. 3. Predominant marker chromosomes compared with the normal chromosomes from the various cell lines (see text).
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