Establishment of a Cell Line (Co-115) from a Human Colon Carcinoma Transplanted into Nude Mice

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Summary

A human colon carcinoma cell line, Co-115, has been established in vitro from solid xenografts maintained in nude mice and subcultured for 95 passages. Co-115 cells grow in vitro as tightly packed, epithelial-like colonies, have a doubling time of about 36 hr, have a relatively low plating efficiency in agar, and release significant amounts of cancer-associated oncoembryonic antigen to the culture medium. Their epithelial nature has been confirmed by ultrastructural examination. The injection of Co-115 cells into nude mice reinduced the formation of solid tumor masses that could be retransplanted and showed a morphology comparable to that of the original xenograft.

Introduction

Cell lines of human colon carcinomas are difficult to establish. Indeed, only a few of them have been reported in the literature (3, 7, 17). One of the major difficulties to overcome in establishing cell lines of human colorectal carcinomas is the bacterial contamination present in the surgical specimens. Furthermore, when primary cultures of colon carcinomas could be obtained, the neoplastic cells were usually overshadowed by fibroblast-like cells in a few months. Yet, cultures of gastrointestinal tumors represent useful experimental material for a variety of studies. In particular, human tumor cells have been shown to possess tumor-associated or -specific antigens, and cell cultures may be a convenient source of malignant material for antigenic studies. Indeed, CEA (6) has been demonstrated in cultures of gastrointestinal neoplasms (2, 7, 17). We wish to report here the establishment and preliminary characterization of an epithelial tumor cell line, referred to as Co-115 and derived from a human colon carcinoma previously grafted and maintained by serial transplantation into nude mice. The human tumor growing in the nude mouse was found to be supported by a stroma of murine origin, and contamination by human fibroblasts was therefore minimal or absent. Isolation of tumor material from nude mice has the advantage of providing sterile material and of allowing the elimination of murine fibroblasts present in the primary culture.

Materials and Methods

Tumor Tissue. The primary tumor was obtained shortly after surgery from a 77-year-old Caucasian female with a carcinoma of the ascending colon. Histological examination revealed a poorly differentiated adenocarcinoma (see Fig. 1A) that invaded the entire thickness of the bowel wall and metastasized to the regional lymph nodes (Duke's C; Duke's classification, which refers to the pathological extent of the neoplasm). Rare gland-like structures were present, with little if any mucin production.

Heterotransplantation. The tumor fragments were minced in culture medium and inoculated s.c. with a trocar into the flanks of the nude mice. Six-week-old nude mice, backcross mated with BALB/c and bred in our colony under conventional conditions, were used in these experiments. For serial passage of the transplants, the tumor nodules were dissected and freed from necrotic tissue before reincorporation. Serial samples were taken for examination by light and electron microscopy.

Cell Cultures. Cell cultures from nodules of different passages were prepared by mincing the tissue with a scalpel into fragments of approximately 2 mm in diameter. The fragments were then suspended in the culture medium and seeded in Falcon plastic flasks (25 sq cm). In addition, a fine cell suspension was prepared by digestion of tumor fragments with 0.25% trypsin for 20 min at room temperature. About 10^6 tumor cells obtained by this procedure were placed in tissue culture flasks of the same size. Dulbecco's modified Eagle's medium supplemented with 20% heat-inactivated FCS (Gibco Bio-Cult Ltd., Scotland) containing 200 µg streptomycin per ml and 200 IU penicillin G per ml was used to initiate the cultures. All cultures were incubated at 37° in 5% CO₂ in air. Subsequently, the cultures were serially transferred by treatment with 0.05% trypsin and 0.05% EDTA and maintained in the same medium containing 10% FCS.

Co-115 cells were viably frozen in medium plus 7.5% dimethyl sulfoxide and were maintained at -80° at different passages over the past 2 years.

Growth Curves and Plating Efficiency. Cultures of human colon carcinoma Co-115 cells were collected after 20 and 50 passages for the determination of growth curves. For each passage studied, semiconfluent cultures were...
trypsinized (trypsin:EDTA), and the cells were washed twice and resuspended in medium with 5% FCS for counting. Three plastic flasks (25 sq cm) were plated with 10^6 cells and counts were carried out at 48-hr intervals up to Day 10. Trypsinized cells were counted in a hemocytometer and their numbers were averaged for each time interval.

The plating efficiency of Co-115 was determined in soft agar according to the method of MacPherson and Montagnier (12). Cells were suspended in growth medium containing 0.3% agar (Difco Laboratories, Detroit, Mich.) and were seeded into 60- x 15-mm Petri dishes containing a base of 3 ml of 0.5% agar in growth medium. Tumor cells were seeded in duplicate dishes at concentrations of 10^3, 10^4, and 10^5 cells. Colony formation in agar was scored after 3 weeks of culture, and colonies with 30 or more cells were counted.

**Reinoculation of Cultured Tumor Cells into Nude Mice.** Cultured Co-115 cells (30 x 10^6) were injected s.c. into nude mice as previously done for other cell lines (5, 8, 9, 13, 15). The tumors that developed were used in part for histological examination and in part for transplantation into other mice. By this technique, several serial passages were carried out.

**Cytogenetic Analysis.** Confluent monolayers of cultures of Co-115 cells were treated with colchicine at a final concentration of 0.08 mg/ml in the medium. After 4 hr, the cultures were dispersed at 37° with 0.25% trypsin and the cells were centrifuged at 800 rpm for 5 min. The cells were then resuspended in a hypotonic solution (1% sodium citrate in distilled water) for 20 min at 37°. Cells were then pelleted and fixed in ethanol:acetic acid (3:1) at room temperature for 30 min. The cell suspension was dropped onto a chilled wet slide (distilled water), air dried, and stained with 2% Giemsa for 20 min.

**CEA Assays.** CEA was identified in 0.6 m perchloric acid extracts of Co-115 tumor heterografts by means of the radioimmunooassay described by Thompson et al. (16) and modified by Mach et al. (11). CEA in the sera of tumor-bearing nude mice, as well as in supernatants of confluent cultures, was determined by means of a modification of the double-antibody radioimmunooassay described by Egan et al. (1). Indirect fluorescent staining of CEA was performed on cryostat sections of a tumor transplant obtained after the 2nd transfer and on monolayers of Co-115 cells grown on coverslips.

**Blood Group Antigens.** The persistence of blood group substance(s) on Co-115 cell membranes was tested by an indirect immunofluorescence technique using human donor sera against blood groups A, B, and O.

**Microscopy.** Tumor fragments for light microscopic examination were fixed with 2.5% cetylpyridinium-buffered glutaraldehyde, embedded in butoxyethanol glycolmethacrylate (Sorvall, Newton, Conn.), sectioned at 2 µm, and stained with Giemsa and periodic acid-Schiff or Azan blue at pH 2.6 reagents.

For electron microscopy, the tissues were fixed with 2.5% glutaraldehyde buffered with 0.1 m cacodylate, pH 7.3, postfixed in 1% osmium tetroxide, and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

Confluent cultures of Co-115 cells (95th passage) growing on a Falcon plastic flask surface were fixed in situ with the use of glutaraldehyde and osmium, as above. The cells were dehydrated and embedded in the flask. Ultrathin sections were performed parallel and perpendicular to the plastic surface.

**RESULTS**

**Heterotransplantation.** Tumor nodules (s.c.) developed in 3 of 3 mice originally grafted and reached a diameter of 5 to 8 mm within 2 to 3 weeks. Up to the present time, 50 successive transplantations have been carried out. The growth rate of the tumors increased during the 1st 4 passages and then remained constant, yielding approximately 3 to 4 g of tumor in 4 weeks.

Microscopic examination revealed that all tumors growing in nude mice were composed of carcinoma cells featuring essentially the same degree of differentiation as the original tumor (Fig. 1B). These cells were arranged in masses supported by a vascularized stroma of murine origin which appeared less abundant than that of the original tumor (B. Sordat, unpublished results, 1974). Necrotic foci were frequently observed in the center of larger nodules. At the periphery, the tumor nodules appeared to be well delimited but not encapsulated. At the electron microscopic level, the tumor transplants consisted mostly of poorly differentiated cells with large nuclei and distinct nucleoli. Their cytoplasm was rich in polysomes and contained numerous mitochondria but rare profiles of ergastoplasm and of Golgi complexes. The intercellular spaces presented frequent interdigitations and microvilli, as illustrated in Fig. 2A. Cell junctions between the tumor cells were mainly of the desmosome type. The fine structure of these neoplastic cells did not change in subsequent passages. No basal lamina was ever recognized at the periphery of the tumor nodule, although some amorphous electron-dense material was occasionally juxtaposed with the tumor cells. No virus particles have been seen in association with the tumor cells.

**Cell Cultures.** Primary cultures were established more easily from later than from earlier transplants. These cultures were characterized by colonies of cells featuring an epithelial pattern of growth after 2 to 5 days of explantation. The epithelial colonies consisted of relatively slow-growing cells showing pleomorphism and some vacuolization. These colonies increased in size for 3 to 4 weeks. As they grew larger, piling up of cells occurred in their center. Fibroblasts of murine origin were present in variable numbers. When tumor cells from early passages were used, every attempt to subculture these slow-growing epithelial cells was unsuccessful. The remaining cells being of mouse origin. In contrast, when tumor cells from the 10th to 15th passages in the mouse were used, the colonies consisted of more rapidly growing epithelial cells which could be easily subcultured. Cell line Co-115 was established in October 1974 from such colonies that demonstrated a strong adherence to the plastic surface and grew in tightly

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*The murine origin of the stroma within transplanted tumors has been demonstrated by indirect immunofluorescence with antispecies antisera on cryostat sections of colorectal xenografts, including Co-115 tumor.*

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**Colon Carcinoma Cell Line**

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packed, epithelial-like formations (Fig. 1C). The tumor cells were separated from the mouse fibroblasts by repeated short periods of trypsinization (3 times at 2-day intervals), during which most of the fibroblast-like cells could be removed by gentle shaking. This procedure proved to be very successful in yielding almost pure colonies of tumor cells which grew to confluence. The remaining fibroblasts were lost during successive subcultures. In addition, cytotoxicity tests performed on ⁶⁰Cr-labeled Co-115 cells in the presence of antitumor antiserum dilutions plus complement did not reveal a significant radioactive release. These results strongly indicate the disappearance and elimination of murine cells in the Co-115 cultures. Moreover, the tumor cells had essentially the same morphological appearance as did the primary cultures and showed some mucin production.

The fine structure of the cultured cells was remarkably similar to that of the tumor cells described for the solid transplants. Sections parallel to the surface of the monolayer showed that the cells were in close contact with each other and presented numerous cell-to-cell junctions of the desmosome type. The intercellular spaces were often focally dilated, forming “intercellular lumens” containing numerous microvilli (Fig. 3A). Desmosomes were sometimes present at both sides of these spaces. Rare lumens entirely surrounded by cytoplasm were occasionally seen; it could not be established, however, whether they were entirely intracellular or whether they represented extensions of the extracellular spaces. The microvilli at the surface of the cells were generally numerous and displayed a core of fine filaments extending into the cytoplasm in the form of deep rosettes. Filaments were present in all cells, the larger ones (80 to 120 Å in diameter) arranged in bundles sometimes converging toward desmosomes, and the finer ones (40 to 80 Å) forming a felt-like meshwork. Small vesicles were present along the plasma membrane of most cells. In some of them, membrane-bound granules containing material reminiscent of mucin were also observed (Fig. 3B).

**Growth Curves and Plating Efficiency.** Co-115 cell cultures first showed an initial growth lag of about 5 days, then an exponential phase of growth between Days 5 and 10 after plating. The mean population doubling time was about 56 hr when tested between Passages 8 and 15 and was about 36 hr after the 26th passage. The mitotic index, counted on cells 7 days after the 80th in vitro passage, varied between 0.9 and 1.2%. The plating efficiency was determined with the same cell preparations that were used to establish the growth curves. The colonies were counted 3 weeks after plating. The absolute plating efficiency of Co-115 cell line at Passage 34 was 3%. This result was relatively low, compared with another colon carcinoma line, HT-29 (initially cultured by Dr. J. Fogh of the Sloan-Kettering Institute for Cancer Research), which exhibited, under similar culture conditions, a plating efficiency of 13%.

**Tumorigenicity in Vivo.** Co-115 cells (30 x 10⁶) were injected s.c. into 3 nude mice, all of which developed a tumor after a latency period of 3 to 4 weeks. These tumors were easily retransplantable into nude mice where they reproduced a morphology comparable to that of the solid transplants (see Fig. 1D).

**Blood Group Antigens.** Among the different human sera tested against the ABO blood group substances, only the anti-A serum gave a distinct positive fluorescence, which confirmed the donor’s known blood group.

**CEA Assays.** Co-115 cells produced significant quantities of CEA in vitro, as well as in heterotransplants. Culture medium of confluent monolayers contained as much as 300 ng CEA in 10 ml medium after 5 days of culture.

CEA was also demonstrated in the circulating blood of nude mice bearing large tumors. In these instances, serum concentrations of CEA reached values up to 118 ng/ml. Extracts of Co-115 heterografts gave 1.5 to 6.3 μg CEA per g of wet tumor tissue.

Demonstration of CEA by indirect fluorescence on cryostat sections of Co-115 tumor nodules at the 2nd transfer revealed a localization in the intercellular spaces while, on Co-115 monolayers grown on coverslips, CEA was shown to be localized on the membrane of the tumor cells.

**Cytogenetic Analysis.** Karyotypic analysis was performed on Co-115 cells from the 50th passage. Among 8 metaphases that could be analyzed, 7 exhibited a near-diploid mode of 49 chromosomes and 1 had 46 chromosomes. The 3 additional chromosomes of the 7 abnormal karyograms were classified as an extra A, 1 related to the B group, and an extra E or F chromosome. No marker chromosomes for HeLa cells were found in this material. A karyogram, represented in Fig. 2B, illustrates these preliminary results.

**DISCUSSION**

The present report describes the establishment of cell line Co-115 from a human colonic adenocarcinoma. This line, which was obtained from solid heterotransplants maintained in nude mice, grows in culture as tightly packed colonies of epithelial-like cells adherent to the plastic surface of the flask. Ultrastructural examination of the cultured cells confirms the epithelial nature of the Co-115 cell line. Furthermore, the morphology of microvilli and the appearance of secretory material are very comparable to those of intestinal epithelial cells. The mouse fibroblasts present initially could be removed from the cultures after several passages in vitro. The tumors induced in nude mice by s.c. injection of Co-115 cells grown in vitro for 8 months were histologically comparable to the original malignant tumor, although with a less abundant stroma. Similarly, Goldenberg et al. (7) succeeded in establishing a mucin-producing human colon carcinoma line, GW-39, from a solid xenograft growing in the cheek pouch of adult golden hamsters. In this case, fibroblast-like cells admixed with the tumor cells died off after about 3 months of culture. Moreover, 2 permanent cell lines (END-1 and END-2) from poorly differentiated endometrial carcinomas maintained in the nude mouse could also be established in vitro in this laboratory (13). This report raises the question of the importance of the nude mouse as an intermediate host for the establishment of long-term human neoplastic cell lines. Previous attempts in this laboratory to establish cell lines from colon carcinomas taken directly from surgical specimens have been unsuccessful, and a recent report (14) suggests that, if 5 to 10% of the various human tumors initially placed in culture pro-
vided an epithelial type of growth, less than 1% may survive as continuous cell lines. Our experience suggests that the establishment of line Co-115 was facilitated by previous passages of the tumor cells through nude mice. This is further supported by the fact that several other human tumor cell lines were established in this laboratory from heterotransplanted human tumors (1 renal cell carcinoma, 4 malignant melanomas, and 1 moderately differentiated colon carcinoma). The synthesis of CEA and its release in the culture medium by human tumor cells have been previously reported by several authors. Egan and Todd (2) showed, with colon carcinoma line HT-29, that CEA could be detected in the medium after 5 days of culture and that its concentration increased up to 2 months after initial transfer. CEA has also been demonstrated in the supernatant from a lysate of COLO 16, a human squamous carcinoma line (14), and in the culture fluid of Me160, a cervical carcinoma cell line (2). In addition, Tompkins et al. (17) reported the establishment of 2 cell strains derived from colorectal neoplasms HCT-8 and HRT-18, both of which synthesized CEA in vitro. The Co-i15 cells retained the ability to produce CEA in vivo (10) and also in vitro in amounts comparable to those of the HCT-8 and HRT-18 cell strains. Therefore, this line may be useful in further investigations of the possible antigenic differences of CEA molecules produced by these various strains. Moreover, it also retained the capacity to produce another human oncofetal antigen, referred to as BOFA in a recent publication (4). As a source of malignant target material, it may also be used in in vitro studies of humoral and cell-mediated immunity in patients with colon carcinomas.

ACKNOWLEDGMENTS

We are grateful for the excellent technical assistance of J. Bamat, L. Kolly, and S. Salvi in these studies. We thank Dr. M. Jotterand-Bellomo, Department of Medical Genetics, University Hospital of Lausanne, for performing the karyotypic analysis, as well as Professor C. Bron, Institute of Biochemistry, University of Lausanne, for his gift of antispecies antisera. We also thank Professor L. Ozzello, Professor K. T. Brunner, and Professor J.-P. Mach for suggestions and advice in the preparation of the manuscript.

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