Marker Properties of Tumor and Lymphoid Cell Lines Derived from a Patient with Squamous Cell Carcinoma

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SUMMARY

A squamous cell carcinoma tumor cell line, COLO 227, derived from a metastatic tumor in a Caucasian male, produces both parathyroid hormone and carcinoembryonic antigen. A chromosome mode of 106 predominated and the X and Y chromosomes were retained. Seven marker chromosomes were identified. Cytogenetic analyses revealed an isochromosome 17 marker described in other cancers. An autochthonous lymphoid cell line, COLO 219, was established and characterized. COLO 219 is a normal lymphoid cell line with B-cell characteristics. This autochthonous system of both cultured tumor cells and cultured lymphocytes is of use in immunological studies.

INTRODUCTION

A 59-year-old Caucasian male had severe burns to both legs many years previously. The burned areas repeatedly ulcerated and were treated by many skin grafts. In January 1975, he was found to have undifferentiated squamous cell carcinoma of the left ankle. Unfortunately, 2 days previously, a cross-leg pedicle graft was connected from the right-upper thigh to the left ankle. The graft was removed and replaced at the original site and a left above-the-knee amputation was performed. In April 1975, a carcinomatous implant at the site of the pedicle graft was widely excised. A recurrence of this right-thigh metastatic lesion appeared in June 1975, and 2 pulmonary nodules were noted on X-ray.

This clinical syndrome of an epidermal carcinoma developing at a site of a thermal injury is related to the kangri burn cancer seen in India and the kairo burn cancer of Japan. A similar carcinogenic process called a Marjolin's ulcer develops at the site of chronic draining sinuses. On August 5, 1975, the right upper-thigh lesion was widely excised and the metastatic tumor sample was submitted to us for study. A peripheral blood sample was obtained and cultured on August 8, 1975. The patient was an active, relatively healthy man without abnormal laboratory findings or symptoms of any endocrine disorder.

MATERIALS AND METHODS

Cultures of the squamous cell carcinoma tissue were initiated by mincing the tumor sample in 2-ml volumes of F-12 medium (8), RPMI 1640 (20), GEM 1717 medium (33), and Medium L-15 (12); each was supplemented with 20% FCS (heat-inactivated at 56° for 30 min) (Reheis Chemical Company, Kankakee, Ill.), penicillin (100 units/ml), and streptomycin (50 μg/ml). The tissue suspensions were seeded to 4-oz flint-glass culture bottles. The bottles were loosely capped and incubated at 37° in humidified air with 10% CO₂. Weekly, 1 to 2 ml of fresh medium were added to each culture bottle until cell growth stabilized.

Cultures of peripheral blood leukocytes were initiated by the method described by Moore et al. (20) for establishing lymphocytes. The lymphocyte cultures were initiated in 4-oz flint-glass bottles with RPMI Medium 1640 with 20% FCS, and GEM 1717 medium with 20% FCS.

Serial morphological observations were made of subcultures growing on coverslips by phase microscopy. The cell line was prepared for electron microscopy by the manner described by Moore et al. (21).

Suspensions of the cultured tumor cells for chromosome studies were prepared by scraping the monolayer of 4-day-old subcultures with a rubber-tipped rod. These suspensions were treated for 5 hr at 37° with 0.1 μl Colcemid (Ciba Pharmaceuticals, Summit, N. J.) per 10 ml media. The procedure of Lubs et al. (14) was followed for metaphase preparation by treatment of the cells for 12 min with 0.075 M KCl. The method of Seabright (30) was used for Giemsa banding, and the method described by McKenzie and Lubs (16) was used for constitutive heterochromatin banding.

Cell suspensions from rapidly growing lymphocyte cultures were harvested and treated for 45 min at 37° with 0.02 μg Colcemid per 10 ml media. The lymphoid cells were treated for 9 min with 0.075 M KCl and subsequent metaphase spreads were both Giemsa and constitutive heterochromatin banded.

Spent RPMI Medium 1640 with 10% FCS recovered after 10 days from actively growing tumor cell cultures was assayed for PTH by Dr. S. Krutzik, Nichols Institute, San Pedro, Calif. A CEA radioimmunoassay kit (Roche Diagnostics, Nutley, N. J.) was utilized to assay both 16-day-old spent RPMI Medium 1640 with 20% FCS from a culture of 2.8 x 10⁶ total live cultured tumor cells, and the supernatant from freeze-thaw lysed cells of the same culture. In both instances, assays were done on complete media without exposure to the cells.

The abbreviations used are: RPMI, Roswell Park Memorial Institute; GEM, George E. Moore; FCS, fetal calf serum; PTH, parathyroid hormone; CEA, carcinoembryonic antigen; G6PD, glucose-6-phosphate dehydrogenase. The cytogenetic abbreviations are according to the Paris Conference Nomenclature (1971).
ACTH assays were performed by J. Gassen, University of Colorado Medical Center, Denver, Colo., on 14-day-old spent RPMI Medium 1640 with 10% FCS from lymphocyte cultures and tumor cell cultures. In addition, 6.7 × 10^6 live lymphocytes and 7.4 × 10^6 live tumor cells were assayed for ACTH.

G6PD isoenzyme mobility was assayed with a cellulose-acetate electrophoresis kit obtained from Helena Laboratories, Beaumont, Texas. Cultured lymphocytes (10^7) and cultured tumor cells (10^7) were lysed by freeze-thaw in 1 ml of 0.9% NaCl solution and the supernatant was assayed for G6PD isoenzymes.

Transplantation antigens (HLA) of the cultured lymphocytes were determined by cytotoxicity assay (22). Surface immunoglobulin studies were performed by immunofluorescence as previously described (17).

Insulin and glucagon receptor concentrations were measured on both the cultured lymphocytes and tumor cells by our associate, K. Stedman.

Studies of erythrocyte and erythrocyte-antibody-complement rosettes using sheep RBC were performed on the cultured lymphocytes by a modification of the method of Jondal et al. (10). In addition, the cultured lymphocytes were rosetted with monkey RBC (Macaca speciosa), according to the method of Pellegrino et al. (28), to study B-cell characteristics.

Cytotoxicity assays using COLO 219 as effector cells against COLO 227 target cells at a ratio of 20:1 were performed as previously described (18).

The cultures of lymphocytes and tumor cells were monitored for pleuropneumonia-like organism contamination by inoculating agar and broth as previously described (21), and by the orcein stain technique described by Fogh and Fogh (4).

For storage of both the lymphoid and tumor cultures, cells were suspended in cold RPMI Medium 1640 containing 20% FCS, 10% dimethyl sulfoxide, penicillin, and streptomycin, frozen slowly, and stored at −85°C (21).

**RESULTS**

**Establishment.** The squamous cell carcinoma tumor cell line was designated COLO 227 on November 7, 1975, after the monolayer outgrowth in F-12 medium supplemented with 20% FCS reached semiconfluence and was successfully subcultured several times. Primary cultures initiated in other media formulations failed to establish. COLO 227 has been successfully propagated in several media: F-12, RPMI Medium 1640, and GEM 1717 medium, each supplemented with either 10 or 20% FCS. For subculture, cells were dispersed by either shaking or scraping the monolayer and seeding the resultant cell suspension in some spent medium in addition to fresh medium. Successful subcultures were split 1:2 at 2-week intervals for over 1 year.

The autochthonous lymphocyte line cell line, obtained from peripheral blood and established in RPMI Medium 1640 with 20% FCS, was designated COLO 219 on November 7, 1975. Signal for establishment of the lymphoid cell line was the appearance of cell clumps in the supernatant, rapid cell growth, and successful subculture. COLO 219 has been subsequently maintained in both RPMI Medium 1640 and GEM 1717 medium each supplemented with either 10 or 20% FCS. Morphological studies revealed lymphoid cells, and growth curves indicated doubling times of 20 to 24 hr.

**Cell Line Characteristics.** The cultured squamous carcinoma cells grew in compact colonies on the glass and had a uniform epithelial appearance (Fig. 1). As reported with other squamous cell carcinoma cell lines (21), subcultures had a uniformly low mitotic activity and remained as scattered clusters of cells for weeks before confluent growth was achieved. COLO 227 cells were polygonal in shape and ranged in size from 29 to 31 μm. Occasional binucleated giant cells, which probably represented fused cells, were observed. Conspicuous "intercellular bridges" spanned wide intercellular spaces. The vesicular nuclei were irregularly shaped with large prominent nucleoli. Numerous small refractile vesicles were present in the perinuclear region of the cytoplasm.

Electron microscopic examination of COLO 227 showed round and polygonal cells with elaborately convoluted cellular membranes that formed villous projections which interdigitated with those of adjacent tumor cells (Fig. 2). Tonofibrils were present in most of the tumor cells as bundles of intermeshed filaments which anastomosed with each other and converged toward well-developed desmosomal attachments. The mitochondria showed considerable variation in size, shape, and density of the matrix. Free ribosomes were present, along with extensive rough endoplasmic reticulum. Prominent Golgi apparatus was present in most cells. Numerous cytoplasmic vesicles were common, although no phagocytic activity of dead or dying cells was noted.

Chromosomal analyses of COLO 227 were made from 61 metaphase spreads, and 37 of these spreads were photographed. Approximately 80% of the metaphases analyzed had between 105 and 108 chromosomes with a mode of 106. Four copies of the normal complement of chromosomes predominated with only slight variation. In all metaphases analyzed, the X and Y chromosomes were present. No polymorphisms were noted.

The markers were identified as follows (Fig. 3):

- M1: del(1)t(1;?:)(1q12—>1p36::?)
- M2: i(17)(qter—cen—qter)
- M3: i(8)(qter—cen—qter)
- M4: t(1;3)(1qter—cen—Spter)
- M5: t(X;17)(Xqter—cen—17qter)
- M6: i(9)(qter—cen—qter)
- M7: dic(19;20)(19qter—19p13::20q13—20pter)

The IQ portion of markers M4 and M6 were verified by constitutive heterochromatin banding. Markers M1 through M7 were present in greater than 85% of the metaphases analyzed with M1, M2, and M3 present in all cells studied. Markers M4 and M5 were present in 50 to 85% of the metaphases. Six unidentifiable abnormal chromosomes of unknown origin were occasionally observed.

Chromosomal analyses of autochthonous lymphoid cell line COLO 219 were made from 97 metaphase spreads, and 22 of these spreads were photographed. The modal number of chromosomes was 46. Analyses of metaphases with less than 46 chromosomes indicated a random loss of different chromosomes. Polyploid metaphases were observed in
5.2% of the lymphocytes. All metaphases analyzed had the X and Y chromosomes present. The chromosomes of COLO 219 (Fig. 4) were consistent with a normal male karyotype. No polymorphisms were noted with constitutive heterochromatin banding.

The HLA profile of COLO 219 was A2, B8.

**Cell Products.** The level of PTH in spent RPMI Medium 1640 with 10% FCS from COLO 227 was 42 μl Eq/ml as compared to undetectable amounts for the control fresh medium. This observation is consistent with a previously noted squamous cell carcinoma cell line with significant ectopic PTH production (21).

Analysis of the freeze-thaw lysate supernatant of COLO 227 for CEA showed a concentration of 0.4 ng/10⁶ live cultured tumor cells and 4.9 ng/ml in the spent medium.

ACTH production was negative in both spent media and live cells of COLO 227 and COLO 219.

Assays for G6PD isoenzyme mobility performed on lysates of COLO 227 and COLO 219 were positive for type B.

Assays for insulin and glucagon receptor sites on the cell surface of COLO 219 revealed that each lymphocyte bound approximately 328 molecules of ¹²⁵I-labeled insulin and no molecules of ¹²⁵I-labeled glucagon. No insulin or glucagon receptors were detected on the tumor cells (COLO 227).

COLO 219 contained cell populations positive for IgG, IgM, IgD, IgA, and IgE, in addition to C’3. COLO 219 was negative for both erythrocyte and erythrocyte-antibody-complement rosettes, but 97% of the cells formed spontaneous monkey RBC rosettes.

COLO 219 cells achieved 100% destruction of COLO 227 target cells in 96 hr as measured in the visual cytotoxicity assay.

In cultures of both COLO 219 and COLO 227, no pneumopenia-like organism contamination was detected by the broth and agar techniques or the orcein cytochemical method.

**DISCUSSION**

Ectopic PTH production by various neoplasms of nonparathyroid origin has been previously reported (5, 27). Squamous epithelium cancers of the respiratory tract are considered the most frequent sources of PTH (24). Burn scar carcinoma (6), squamous cell carcinoma of the mouth (31), and epidermoid carcinoma of the penis (29) also have been shown to induce hyperparathyroidism.

Tashjian (32) reviewed the production of PTH by tumor cells in culture. We have previously described a squamous cell carcinoma tumor cell line (COLO 16) that produced PTH in culture (21). COLO 227 also produces significant amounts of PTH after more than 1 year in culture, although the donor had no history of hypercalcemia or hyperparathyroidism.

Some cultured human tumor cell lines are reported to synthesize CEA (1, 7, 21). COLO 227 elaborates appreciable amounts of CEA in the culture medium, while smaller amounts remained within the cells.

It may be significant that both COLO 16 and COLO 227 were derived from metastatic burn scar tumors and both produce CEA and PTH.

Cytogenetic analyses of COLO 227 revealed a stable cell line with 5 identifiable markers present in greater than 85% of the metaphases analyzed. Both X and Y chromosomes were retained in the tumor karyotype. Isochromosomes represented 3 (M₁, M₂, and M₃) marker chromosomes, and chromosome 17 was involved in 2 (M₄ and M₅) markers.

Three markers of COLO 227 are either similar morphologically or have similar breakpoints to markers described in other cancers. A deletion breakpoint at q12 observed in M₁, of COLO 227 was also reported in 2 lung carcinomas, as reported by Kakati et al. (11). The markers differ in that M₁ of COLO 227 has a terminal translocation. Similarly, another cell line, Lu106, analyzed by Heenen (9), had several markers common with HeLa cell lines and a marker with a deletion 1 breakpoint as in M₄ of COLO 227. An isochromosome 8 similar to M₅ of COLO 227 has been identified (9) in 2 long-established cell lines, Hep-2 and KB, which have been indicted as HeLa contaminated (9, 13, 23). Also, an isochromosome 8 was depicted by O'Toole et al. (26) in a squamous cell carcinoma cell line of urinary bladder origin (SCaBER). An isochromosome 17 similar to M₆ of COLO 227 has been described in a metastatic prostate carcinoma (11), ovarian tumor (11), prostatic cancer (25), myeloid leukemia (2), Sézary syndrome (15), eosinophilic leukemia (19), and malignant lymphoma (3).

Although a few markers of COLO 227 were similar to those reported in other cancers and established neoplastic cell lines, too few tumor cell lines have been characterized cytogenetically in the literature to make any firm conclusion concerning the nonrandom involvement of chromosomes in these cancers.

COLO 227 is a unique cell line based on its composite of marker chromosomes and cell products. COLO 227 is not a HeLa or HeLa-contaminated cell line, since it was derived from a Caucasian male, the Y chromosome is retained in the cultured tumor cell karyotype, the G6PD mobility is type B variant, and HeLa cells and indicted HeLa cell lines have never been cultured in this laboratory.

The ultramorphology of COLO 227 revealed tonofibrils and desmosomes. Retention of these phenotypic traits for epithelium supports the observation that the origin of COLO 227 was a squamous epithelium cell type.

COLO 219 appears to be a normal lymphoid cell line with B-cell characteristics. The lymphoblast-like cells produced immunoglobulins and did not rosette spontaneously with sheep RBC, but did rosette spontaneously with monkey RBC. The concentration of insulin and glucagon receptors per COLO 219 cell was within the range found for normal lymphocytes.

Mitchen et al. (18) reported the use of cultured lymphoid cells as effectors against autochthonous cultured tumor targets. In similar experiments, COLO 219 effector cells reacted against COLO 227 target cells in cytotoxicity assays at a ratio of 20:1 and achieved 100% kill of the targets in 96 hr.

COLO 219 and COLO 227 have been recovered from our cell bank after "slow freezing." They are available to other investigators for their studies.

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REFERENCES


Fig. 1. Morphology of COLO 227 with phase contrast photography. × 400.
Fig. 2. Ultrastructure of COLO 227 showing villous projections. × 3300
Fig. 3. COLO 227 Giemsa-banded marker chromosomes.
Fig. 4. Giemsa-banded karyotype of COLO 219.
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