Tumor Antigen and Human Chorionic Gonadotropin in CaSki Cells: A New Epidermoid Cervical Cancer Cell Line

Abstract. *Epidermoid cervical carcinoma cells (CaSki line) have been established in continuous culture. When leukocytes from cervical cancer patients were incubated with CaSki culture fluid concentrates, inhibition of leukocyte migration was observed in more than 70 percent of the patients tested. By contrast, significantly less inhibition was observed with normal donor leukocytes or leukocytes from patients with other types of cancer. These results were consistent with the expression of tumor-associated antigen by CaSki cells. Analysis of the serum from the donor of the cell line at the time of tumor biopsy, and of CaSki culture fluids, demonstrated the presence of the beta subunit of human chorionic gonadotropin.*

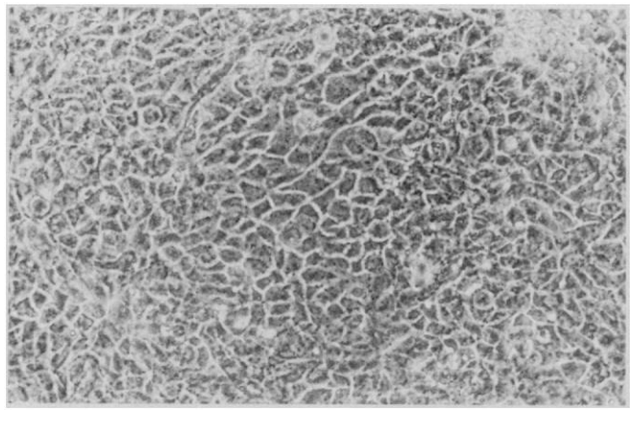
Retrodifferentiation and reexpression of fetal or embryonic genes is well recognized in the reappearance of carcinoembryonic antigen, alpha fetoprotein, and other markers in cancer (*1*). The placental trophoblast cell appears at the first order of differentiation in the two-cell stage occurring approximately 30 hours after fertilization of the human ovum (*2*). Human chorionic gonadotropin (hCG), a major pregnancy hormone, can be detected in the serum of the gestational female within the first week of conception (*3*).

Trophoblast gene derepression resulting in the ectopic production of hCG or its subunits has been considered as a basis for the nonendocrine production of this pregnancy hormone in several forms of malignancy (*4*). In a study of the serums of 1319 cancer patients, hCG production was significant in many cancers (*5*). In this group, hCG was observed in 24 percent of ovarian cancers, 17 percent of gastrointestinal tract cancers, and 33 percent of pancreatic cancers. Production of hCG and its subunits in tissue culture by cell lines derived from lung cancer [ChaGo line (*6*)] and cervical adenocarcinoma [HeLa cells (*7*)] has been reported.

We now report the establishment of the CaSki cell line from an epidermoid carcinoma of the human cervix. Secretion of hCG-β by the established cell line and the presence of hCG (or hCG-β) in the serum of the patient from whom the tumor was derived is reported. In addition, we describe the ability of soluble preparations obtained from CaSki culture fluids to inhibit migration of leukocytes from epidermoid cervical cancer patients, but not generally from heterologous cancer patients or normal individuals.

The CaSki cell line was derived from epidermoid carcinoma of the cervix metastatic to the small bowel mesentery. The patient was a 40-year-old Caucasian female who had previously undergone irradiation and surgical treatment of the malignancy. One year later, because of recurrent cancer, surgery was performed, and a fragment of the tissue from this operation was initiated in explant culture. A blood sample from the patient at that time revealed 44 international units of hCG per liter by radioimmunoassay in the homologous hCG assay (this assay also recognizes hCG-β). By means of two No. 11 scalpel blades under binocular magnification, the tumor was sharply incised into 0.1-mm fragments in a petri dish and explanted with curved Pasteur pipettes into Falcon plastic flasks (25 cm^2). Monolayer tumor epithelial growth was observed within 1 week, and 1 week later 0.2-mm fragments were removed with Pasteur pipettes and mechanically transected into smaller fragments, along with fragments of the accompanying fibroblast growth that served as a feeder layer. Fibroblast proportions were gradually decreased as epithelial fragments were increased. At the point when tumor epithelial cells were predominant, subculture could be successfully carried out with enzyme dispersion, with a solution of 0.01 percent trypsin and 0.1 m*M* EDTA. Ultimately, a pure epithelial population of tumor cells was derived (Fig. 1). The cells continue to show histologic evidence of keratinization in culture as in the original surgical specimen and show desmosomes between the epithelial cells on electron microscopy (not shown). The cells are not contact-inhibited and display the tendency to pile up and form multilayers. The chromosomes are aneuploid, showing a range of 66 to 95 with a modal number of 77 and lesser frequencies of 68 and 85 (Fig. 2). Unlike HeLa cells, isoenzyme mobility patterns for glucose-6-phosphate dehydrogenase (G6PD) of CaSki cells were of the slow-moving B type. Preliminary Q and G banding studies suggested unique chromosomal markers (*8*). Examination indicated that the cells were free of mycoplasma.

Tumor-associated antigen was recovered from the medium in which these cells were grown. CaSki cells were grown on medium consisting of 50 percent Waymouth's MB 752/1, 40 percent Gey's balanced salt solution, and 10 percent fetal calf serum (Gibco). Five days after subculture, cells were washed three times with Gey's balanced salt solution and placed on RPMI 1640 medium without serum. After 24 hours, the culture fluid was removed and centrifuged at low speed to remove nonadherent cells. The medium was centrifuged at 105,000*g* for 90 minutes (Beckman model L2-65B, SW-27 rotor). The supernatant was concentrated by molecular filtration with a stirred cell apparatus equipped with a



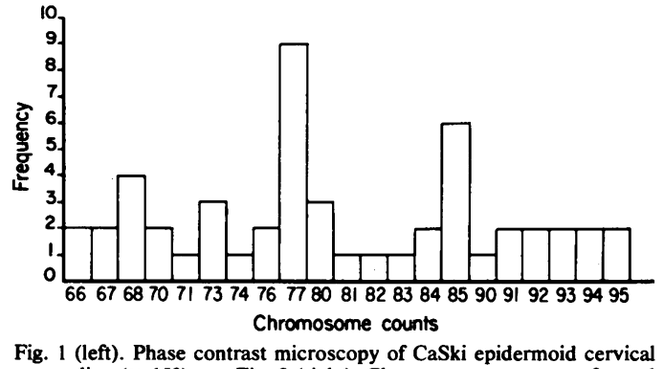


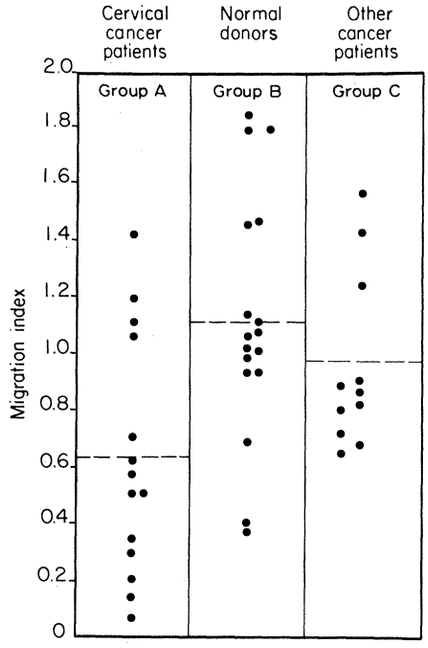
Fig. 1 (left). Phase contrast microscopy of CaSki epidermoid cervical cancer line (× 150). Fig. 2 (right). Chromosome counts performed on 50 CaSki spreads.

type UM 20 membrane (Amicon). The CaSki cells were returned for 2 days to medium containing serum, washed, and placed on serum-free medium. A second antigen harvest was then made after 24 hours. The concentrate was centrifuged at 12,000 rev/min (Sorvall) and sterilized by filtration (0.22 μm; Millipore). Protein was assayed (*9*) after acid precipitation (*10*).

The method used for verification of the presence of tumor-associated antigen in the chemically defined media in which the CaSki cells were grown was the antigen-induced leukocyte migration inhibition assay (*11*). Briefly, heparinized whole blood was allowed to settle at 37°C for 1 to 2 hours. The plasma layer containing the leukocytes was sedimented, and the leukocytes were treated at 4°C for 10 minutes with 0.83 percent ammonium chloride to lyse the red cells. The cell pellet was washed twice with medium consisting of 50 percent Waymouth's MB 752/1, 40 percent Gey's balanced salt solution, 10 percent heat-inactivated fetal calf serum, penicillin (50 unit/ml) and streptomycin (50 μg/ml). The leukocytes were counted and adjusted to 4×10^7 to 6×10^7 cell/ml. CaSki antigen was added to the leukocyte suspension to a final concentration of 700 to 750 μg/ml and incubated for 45 to 60 minutes at 37°C in an atmosphere of 5 percent CO_2 in air. Leukocytes of the same source were incubated without antigen serving as control. The cell suspension was drawn into 25-μl capillary pipettes. One end was closed with sealing clay, and the pipettes were centrifuged at 200*g* for 10 minutes. Capillaries were cut just below the interface between cell and liquid and placed in migration chambers. The chambers were filled with medium and sealed with paraffin. After 18 to 24 hours of incubation, the area of migration was projected onto Whatman 31ET paper and determined by proportional weight. The migration index (MI) is the ratio of the mean of migration of three to four replicates in the presence of antigen to the mean of migration in three to four replicates in the absence of antigen.

In accordance with criteria established by McCoy *et al.* (*11*), leukocytes were considered to be inhibited in migration if an MI of less than 0.8 was observed. In addition, data were analyzed by the paired Student *t*-test; MI values less than 0.8 but not significant ($P > .05$) by the *t*-test were discarded and, when possible, the individual was retested.

Figure 3 illustrates data from the direct leukocyte migration inhibition assay. Leukocytes from 17 cervical cancer patients, 17 normal donors, and 11 patients with other types of cancer were tested with the CaSki antigen. Leukocytes from 13 cervical cancer patients (76.5 percent) had MI values below 0.8, although in three of these cases the leukocytes were not significantly inhibited when analyzed by the paired Student *t*-test. Since none of the three patients in question was available for retesting, values from these individuals are not included in Fig. 3. Of the 17 normal donors tested, leukocytes from only 3 (17.6 percent) were inhibited. Leukocytes from 3 of 11 patients with other types of cancer (27.3 percent) had MI values significantly less than 0.8. By the Wilcoxon rank



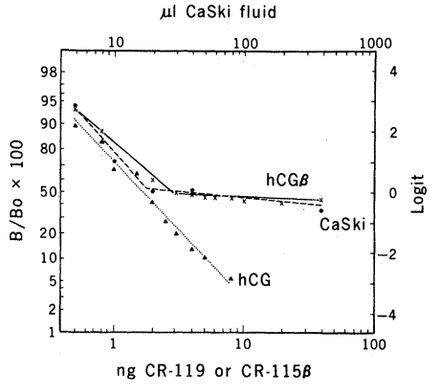


Fig. 3 (left). Migration inhibition assays. Leukocytes were incubated in the presence and in the absence of CaSki culture fluid concentrates. The mean MI ± standard error of the mean for 14 cervical cancer patients (group A) was 0.63 ± 0.11; for 17 normal donors (group B), 1.12 ± 0.11; and for 11 patients with other types of cancer (group C), 0.96 ± 0.09. Group A differs significantly from group B ($P < .01$); group A differs significantly from group C ($P < .05$). Group B and group C were not significantly different. Mean (---). Fig. 4 (right). Dose-response lines for hCG (▲), hCG-β (X), and CaSki culture fluid (●) in the homologous hCG radioimmunoassay. Highly purified hCG (CR-119) and hCG-β (CR-115B) were provided by the Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD). Antibody to hCG (Ortho Laboratories) was used at a final dilution of 1 : 180,000.

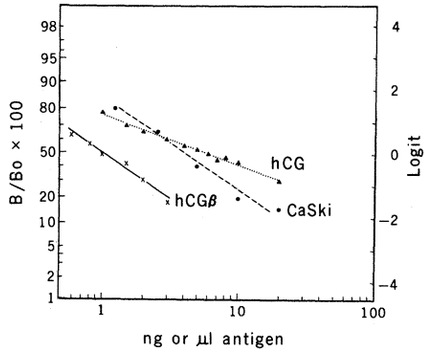


Fig. 5 (left). Dose-response lines for hCG (▲), hCG-β (X), and CaSki culture fluid (●) in the homologous hCG radioimmunoassay. Antibody to hCG-β (hCG-SB6, NIAMDD) was used at a final dilution of 1:50,000. Other details were as described in the legend to Fig. 4.

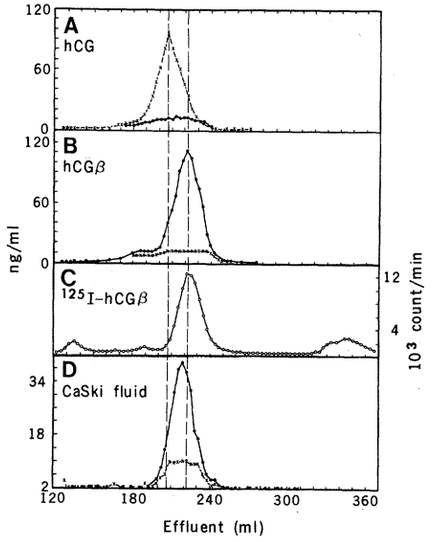


Fig. 6 (right). Sephadex G-200 (2.5 by 64 cm) gel filtration of (A) highly purified hCG (CR-119), (B) highly purified hCG-β (CR-115B), and (D) CaSki fluid containing (C) a trace of ^{125}I-labeled hCG-β as marker. The eluting buffer consisted of 0.01*M* phosphate, 0.14*M* NaCl, and 0.1 percent NaN_3, *p*H 7.5. Fractions (3 ml) were collected by gravity at 4°C with a flow rate of approximately 10 ml/hour. The void volume of the column was 132 ml. Eluate fractions (A, B, and D) were radioimmunoassayed in homologous hCG (X) and hCG-β (●) assays. Correction for the trace of ^{125}I-labeled hCG-β (C) was made by adding a portion of each gel filtration fraction to a radioimmunoassay tube without the labeled hormone as in the routine radioimmunoassay. The radioactive material that precipitated with antibody in these control tubes did not significantly affect the results of the assay.

sum test (*12*), cervical cancer patients (group A) differed significantly from normal donors (group B, $P < .01$) and from patients with other types of cancer (group C, $P < .05$). Group B and group C were not significantly different.

To investigate the possibility that leukocyte migration inhibition may be a result of hCG-β in the CaSki antigen preparations (see below), leukocytes from cervical cancer patients or healthy donors were incubated with highly purified hCG-β (*13*). No inhibition of leukocyte migration was seen with hCG-β at levels comparable to those present in antigen preparations or in patient's serum (not shown). Therefore, it appears that CaSki cells express tumor-associated antigen that can be recovered in concentrates of the culture fluid.

For hormone assays, 5-day double-antibody radioimmunoassays were used (*14*). Concentrates of CaSki culture fluid behaved as highly purified hCG-β when assayed in either the homologous hCG (Fig. 4) or hCG-β (Fig. 5) radioimmunoassays. This indicated that the CaSki cells secreted a substance antigenically identical to hCG-β. The CaSki fluid gave a negative response when assayed in the homologous hCG-α radioimmunoassay (results not included). On the basis of the relative response in the homologous hCG and hCG-β assays, it was determined that the CaSki fluid contained 1 percent or less intact hCG. Gel filtration analysis of the CaSki concentrate (Fig. 6D) resulted in a discrete hCG-β peak that eluted earlier (by one tube) than hCG-β (Fig. 6B) or ^{125}I-labeled hCG-β (Fig. 6C). Similar results were obtained when unconcentrated CaSki culture fluids were analyzed by gel filtration and radioimmunoassay (not shown). The lesser hCG immunoreactivity could be accounted for completely on the basis of the 15 percent cross-reactivity of hCG-β in the homologous hCG radioimmunoassay. The slightly lower elution volume of the CaSki hCG-β peak, as compared to highly purified hCG-β, may reflect minor differences in carbohydrate or amino acid content in the glycoprotein secreted by the CaSki cells.

The presence of hCG (or hCG-β) in the serum of the patient from whom the CaSki cell line was derived, and the production of hCG-β by the tumor cells in culture more than 1½ years later, gives evidence of the apparent irreversibility of the depression that occurred in the production by cervical carcinoma cells of this placental hormone marker. The continuing production of tumor-associated antigen and hCG-β by the tumor cells provides possible tools for the immunodiagnosis and immunotherapy of cervical cancer and for the study of hCG-β synthesis and regulation in malignancy.

R. A. PATTILLO, R. O. HUSSA
M. T. STORY
A. C. F. RUCKERT
M. R. SHALABY
R. F. MATTINGLY

Department of Gynecology and Obstetrics, Medical College of Wisconsin, Milwaukee 53226

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Interspecific Hybridization and Caste Specificity of Protein in Fire Ant

Abstract. *One natural population of fire ant in Texas was found to be a hybrid between* Solenopsis geminata *and* S. xyloni. *Evidence from isozyme studies and breeding experiments is provided to demonstrate interspecific hybridization in ants. In this hybrid population, all worker ants have both parental types of nicotinamide adenine dinucleotide–malate dehydrogenase isozymes, but 95 percent of queens possess only the maternal type.*

In the course of taxonomic studies, students of ants often encounter intermediate variants that might represent interspecific hybrids (*1*, *2*). In most cases, these conclusions are based on morphological characters and, at most, are supported by statistical analysis (*2*). Although Cupp *et al.* (*3*) could produce hybrids of *Solenopsis invicta* and *S. richteri* in a study of forced copulation, their results do not demonstrate that hybridization occurs between natural populations of these two imported fire ant species. We now report another case of interspecific hybridization, in nature, between two ant species, but provide, for the first time to our knowledge, evidence from isozyme studies and breeding experiments. We further demonstrate a case of differential expression of parental genes in different castes of ants.

We have been studying the nicotinamide adenine dinucleotide–malate dehydrogenase (NAD–MDH) isozymes of North American fire ants by means of horizontal starch gel electrophoresis. The banding patterns of this enzyme are monomorphic and species-specific in all North American fire ant species (Fig. 1, samples 1 to 9) (*4*). In the summer of

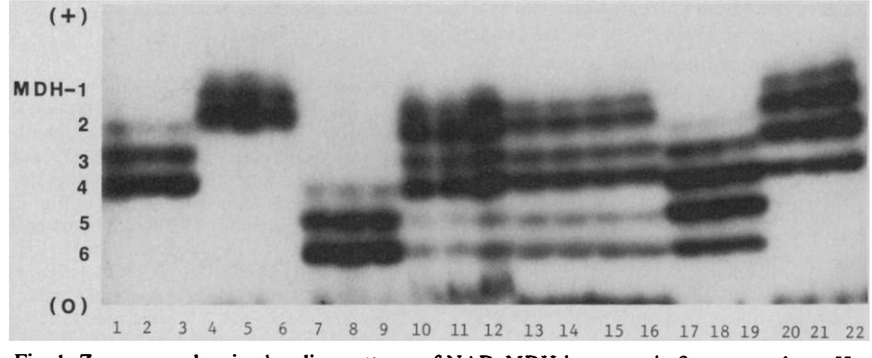

Fig. 1. Zymogram showing banding patterns of NAD–MDH isozymes in fire ant workers. Hybrid samples (10 to 16) have MDH-3 that is not found in parental species. Samples 1 to 3, *S. invicta*; samples 4 to 6, *S. geminata*; samples 7 to 9, *S. xyloni*; samples 10 to 12, *xyloni* × *geminata*, natural; samples 13 to 16, *xyloni* × *geminata*, in vitro; samples 17 to 19, *xyloni* × *invicta*, in vitro; and samples 20 to 22, *invicta* × *geminata*, in vitro.