Cell Line Derived from a Metastasis of a Human Testicular Germ Cell Tumor

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ABSTRACT

A cell line, designated 833K-E, has been established from a metastasis of a human testicular germ cell tumor that consisted of four histological types of tumor cells. The 833K-E cells have morphological and ultrastructural characteristics of epithelial cells and a hyperdiploid karyotype indicative of their human male origin. The cells grow in agar cultures and produce in nude mice tumors which have the histological features of embryonal carcinoma without differentiated elements. Many of the cells express a stage-specific mouse embryonic antigen, and low levels of the major histocompatibility antigens and β2-microglobulin also were detected on a large percentage of the cells. A lymphoblastoid cell line (833K-LC) established from the same tumor specimen expresses major histocompatibility antigens and β2-microglobulin but does not express the embryonic antigen.

INTRODUCTION

Carcinoma of the testis causes 11 to 13% of the deaths from cancer in North American men between the ages of 15 and 34 years, and more than 90% of the testicular tumors in this age group are classified as germ cell tumors (18). Current evidence suggests that these tumors arise from the primordial germ cells, which line the seminiferous tubules and are the direct precursors of sperm.

In the histological classification system proposed by the World Health Organization, testicular germ cell tumors are classified further as EC, teratoma, teratocarcinoma (EC with teratoma), choriocarcinoma, yolk sac tumor, and seminoma (20). EC consists of anaplastic, undifferentiated cells that resemble undifferentiated normal cells of an embryo in the earliest stages of development. Approximately 40% of testicular germ cell tumors contain elements of more than one (e.g., teratocarcinoma), or even all, of the histological types of these tumor cells, and it is thought that the other nonseminomatous germ cell tumors are formed by the differentiation of EC cells (14, 18, 20). Thus, malignant transformation of the germ cells leads to the development of seminoma or, by a separate pathway, of EC, and the EC cells differentiate to form choriocarcinoma, yolk sac tumor, or teratoma. In addition, some teratomas contain cartilage, epithelium, and neural tissue, which suggests the formation by teratoma cells of fully differentiated elements.

Much of the information about these tumors was obtained from studies of mouse testicular teratomas, which were first observed by Stevens and Little (28) and were described in detail by Stevens (25) and by Pierce (22). Testicular teratomas occur spontaneously at high frequency in strain 129 mice and can be induced by grafting genital ridges of 12-day embryos or whole embryos into the testes of syngeneic males (26, 27). These teratomas consist of a variety of tissues representing derivatives of all 3 germ layers.

Some of the induced and spontaneous mouse teratomas can be serially transplanted in syngeneic adult animals. The transplantable tumors also contain EC cells and thus are classified as teratocarcinomas. Several EC cell lines have been established in vitro from these tumors. Some are nullipotential lines, which exhibit little or no tendency to differentiate, whereas others are multipotential lines, which differentiate readily in vitro and in vivo. These EC cells express cell surface (embryonic) antigens that also are detected on undifferentiated cells from other species, including humans, but are not expressed by differentiated cells (reviewed in Refs. 10, 13, and 19).

We are aware of reports of only 4 cell lines, Tera-1, Tera-2, SuSa, and NEC-8, that were derived from human testicular germ cell tumors (9, 11, 30). In a previous communication (4), we described ultrastructural observations of the production, although at low frequency, of particles morphologically identical to the human placental retrovirus (6, 16, 29) by cells (designated 833K-E cells) established in vitro from an abdominal metastasis of a human testicular germ cell tumor. This report describes some additional properties of the 833K-E cell line and compares these properties with those of other human and mouse cell lines of similar classification.

MATERIALS AND METHODS

Clinical History. A right radical orchiectomy was performed in September 1975 on a 19-year-old Caucasian male. Histopathological examination of the testis revealed teratoma, EC, seminoma, and foci of choriocarcinoma. Chemotherapy was initiated with methotrexate, cyclophosphamide, and actinomycin D, but EC was discovered in a left periaortic lymph node in February 1976, and the patient died 2 months later with widespread metastases.

Tissue Culture. Tissue from an abdominal metastasis, consisting of choriocarcinoma with elements of EC, teratoma, and seminoma, was obtained at autopsy. The specimen was placed in culture by the coverslip method (8), and cultures were incubated at 35° in Roswell Park Memorial Institute Medium 1640 containing 10% tryptose phosphate broth, 15% heat-inactivated (56°, 30 min) fetal bovine serum, 2 mM L-glutamine,
100 units of penicillin per ml, and 100 μg of streptomycin per ml.

Cells were detached from culture vessels by incubation for 7 min at 37°C with trypsin:citrate diluted with an equal volume of Grand Island Biological Co. Salt Solution A. Trypsin:citrate contains 0.25% trypsin (Difco Laboratories, Detroit, Mich.; 1:250) in Salt Solution A and, per liter, 10 g of sodium citrate, 5.5 g of sodium chloride, and 0.02 g of phenol red (pH 7.4; formula provided by H.T. Holden, National Cancer Institute). After several passages by this method, cells were subcultured with undiluted trypsin:citrate.

Assays for Mycoplasma were done by in vitro cultivation techniques (Flow Laboratories, Inc., Rockville, Md.) and by ultrastructural examination of the cells. No Mycoplasma were detected.

Tumorigenicity. The methods for seeding cells in agar cultures have been described (7). Athymic (nude) mice were given s.c. inoculations of 5 to 10 x 10⁶ cells, and tumors were removed for histological examination when approximately 5 mm in diameter.

Cyto genetic s. Chromosome analyses were done by conventional staining methods as well as by Q- and G-banding techniques (7).

Antiserum s. Monoclonal antibody W6/32, specific for a determinant common to the 43,000-dalton chains of HLA-A, -B, and -C (2), and monoclonal Antibody B.BM1, reactive with human β₂-microglobulin (3), were kindly provided by Colin Barnstable, University of Oxford. The production and specificity of a monoclonal antibody, anti-SSEA-1, which recognizes a stage-specific mouse embryonic antigen and which reacts with an antibody in the supernatant was assayed by the indirect radioimmunobinding assay described above. The negative controls for these absorptions of W6/32 and B.BM1 antibodies were Daudi cells, which express neither HLA nor β₂-microglobulin (23).

The lymphoid cells were typed for HLA by standard techniques in the Tissue Typing Laboratory, University of Pennsylvania. Expression of HLA alloantigens by 833K-E cells was determined by incubating the cells with 12 μl of alloantisera (Pinquette or O’Driscoll) on ice for 60 min. The cells were then pelleted in a Beckman microfuge, and the residual activity of the serum was titered on peripheral blood lymphocytes from donors of established HLA type [A1, A2, B8; and A3, A11, B12 (B5, B18, BW35)] by the microcytotoxicity assay. Another human testicular tumor cell line, designated 1156Q-E, of known HLA type (A1, A3, B7, B15) was used as a negative control.

For indirect immunofluorescence assays, cells were incubated with antibody and then with rabbit anti-mouse immunoglobulin of appropriate specificity (Cappell Laboratories, Cochranville, Pa.) conjugated with fluorescein isothiocyanate. Incubations were at 4°C in the presence of 0.1% sodium azide. After the cells had been washed 3 times, they were suspended in 50% glycerol in PBS and examined under UV epiillumination with a BP 390-490 violet-blue exciting filter and a No. 515 suppression filter.

RESULTS

Tissue Culture. Numerous small islands of epithelial cells were present in plates and on coverslips a few days after the tissue fragments were placed in culture. Secondary cultures were established with free-floating cells and by transferring coverslips with areas of epithelial cell growth to 35-mm plates. Subsequent passages were made with trypsin:citrate. The cells exhibited scant cytoplasm, large nuclei, and prominent nucleoli (Fig. 1). Ultrastructural examination revealed microvilli, desmosomes, and cytoplasmic tonofibrils (Fig. 2), which are characteristics of epithelial cells. Dome formation, another characteristic of epithelial cells (21), is observed frequently in aged 833K-E cultures. The cells have been subcultured more than 100 times and thus constitute an established cell line.

In addition, lymphocytes persisted in a few of the primary cultures, and proliferation of these cells was noted within 5 to 6 weeks after the tissue was explanted. Each time the medium was replaced in these plates, floating cells were pelleted and seeded in a separate vessel. The continued growth of these cells resulted in establishment of a lymphoblastoid (833K-LC) cell line. The 833K-LC cells grow in suspension (i.e., they do not attach to the vessel growth surface) as single cells or small clusters, are highly pleomorphic, and exhibit uropods. A herpes-type virus was detected by ultrastructural examinations of these cells in early culture, and all cells examined expressed Epstein-Barr virus antigens.® Of the lymphoid cells, the Epstein-Barr virus selectively infects and transforms B-lymphocytes; thus, the expression of these viral antigens suggests that the 833K-LC are derivatives of polyclonal B-lymphocytes (15).

Cyto genetics. Chromosomal analyses were performed with 833K-E cells in passages 5 and 9 with virtually identical results. The cells have a hyperdiploid number of chromosomes,
with a modal number of 56 to 61 in 38 of 58 mitotic spreads counted. Five karyotypes were constructed by G-banding, and 20 mitotic spreads were analyzed. In each of the karyotypes, the A-group chromosomes were overrepresented, whereas the number of chromosomes of the other groups did not deviate consistently from diploid constitution. In all metaphases, 2 or 3 isochromosomes of F-group size were observed, as were 3 to 8 chromosomes of abnormal morphology that could not be identified with certainty. No marker chromosome was found that would identify this cell line specifically. The Y chromosome was detected by Q-banding in all mitotic figures (Fig. 3).

Tumorigenicity. 833K-E cells in passage 85 were inoculated into 5 nude mice. Tumors at least 5 mm in diameter were produced at the site of inoculation in 4 of the animals between 35 and 76 days after inoculation. No metastases were noted. The tumor-free animal died on Day 59 after inoculation. Sections of the tumors from all 4 animals showed cells arranged in tightly packed, convoluted sheets. The tumor cells exhibited a uniform morphology consistent with EC without differentiated elements, although the convoluted areas suggested poorly defined glandular structures (Fig. 4). No tumors formed in 9 nude mice within 44 to 141 days after inoculation (i.e., at the time of death of the animal) with 833K-LC cells.

The 833K-E cells also formed colonies in agar cultures with an efficiency of 1 to 2%. Forty-four clones have been isolated in 3 separate experiments and grown in mass culture for additional characterization studies. The clones exhibit the cellular morphology and growth pattern of the parent 833K-E cell line.

Antigen Expression. Quantitative absorptions of monoclonal antibodies W6/32, B.BM1, and anti-SSEA-1 indicated that cells of the 833K-E line express HLA, \( \beta_2 \)-microglobulin, and the cross-reacting mouse embryonic antigen, SSEA-1. The latter antigen is absent from the 833K-LC cells (Chart 1). Like other lymphoid cell lines, the 833K-LC cells express more HLA and \( \beta_2 \)-microglobulin than do peripheral blood lymphocytes. However, the 833K-E cells express approximately 50% less HLA and \( \beta_2 \)-microglobulin on a per-cell basis than do peripheral blood lymphocytes even though the 833K-E cells are much larger (roughly 5:1 based on the volume of equivalent numbers of packed cells).

Similarly, in immunofluorescence assays, the 833K-E cells showed only a weak, stippled fluorescence after reaction with W6/32 and B.BM1 antibodies, and some of the cells were scored as negative (Table 1). However, the apparent lack of HLA and \( \beta_2 \)-microglobulin on those cells may reflect only the technical difficulties of scoring a weak system. More certain was the observation that some 833K-E cells did not express SSEA-1, because reactive cells fluoresced strongly.

Absorption of HLA typing sera confirmed that HLA of both the A and B loci are expressed by 833K-E and 833K-LC cells (Chart 2). Similar results were obtained with 833K-E cells in passages 34 and 117.

### DISCUSSION

The 833K-E cell line was established from metastatic tumor tissue consisting of 4 histological types of testicular tumor cells, which raises the question of which type(s) of testicular tumor cell is (are) represented by this cell line. As a general observation, it can be stated that the 833K-E cells exhibit ultrastructural and growth properties of epithelial cells and are morphologically similar (i.e., have scant cytoplasm, large nuclei, and prominent nucleoli) when continuously subcultured at a high cell density. All clones derived from this cell line exhibit

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Anti-SSEA-1</th>
<th>W6/32 (anti-HLA)</th>
<th>B.BM1 (anti-( \beta_2 )-microglobulin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>833K-E</td>
<td>67 (+ + +)</td>
<td>92 (+ +)</td>
<td>80 (+)</td>
</tr>
<tr>
<td>833K-LC</td>
<td>0</td>
<td>100 (+ + + +)</td>
<td>100 (+ + +)</td>
</tr>
<tr>
<td>Daudi</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F9</td>
<td>87 (+ + + +)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LNSV(^{a})</td>
<td>0</td>
<td>100</td>
<td>NT(^{c})</td>
</tr>
</tbody>
</table>

\(^{a}\) + + + +, strong fluorescence; +, weak, stippled fluorescence.  
\(^{b}\) Human skin fibroblasts transformed with SV40 (5).  
\(^{c}\) NT, not tested.

**Table 1**  
**Indirect immunofluorescence assays**

Cell suspensions were incubated with monoclonal antibody at 4°C for 60 min, pelleted, washed 3 times, and then incubated at 4°C for 60 min with rabbit antimouse immunoglobulin conjugated with fluorescein isothiocyanate. The cells were pelleted, washed 3 times, and resuspended in 50% glycerol in PBS for examination under UV.

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Chart 1.  
A, absorption analysis of monoclonal antibody (anti-SSEA-1) reactivity. After absorption of antibody by 833K-E (Δ), 833K-LC (○), or F9 (□) cells, residual antibody in the clarified serum was tested by radioimmunoassay on F9 cells as described previously (24). Each point is the average of the results of triplicate assays.  
B, absorption analysis of monoclonal antibody W6/32 (anti-HLA) reactivity on 833K-E (Δ), 833K-LC (○), Daudi (□) cells, or peripheral blood lymphocytes (○) by methods described in A. Residual antibody in the clarified serum was tested on 833K-LC cells.  
C, absorption analysis of monoclonal antibody B.BM1 (anti-\( \beta_2 \)-microglobulin) reactivity on 833K-E (Δ), 833K-LC (○), or Daudi (□) cells or peripheral blood lymphocytes (○) by methods described in A. Residual antibody in the clarified serum was tested on 833K-LC cells.

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These same properties and are morphologically indistinguishable from the parent line. Chromosomal analyses demonstrated that the cells are of human male origin, and, although the chromosomal constitution is grossly abnormal, it is stable, as is indicated by analyses of cells in widely different passages.

The tumors produced by inoculation of 833K-E cells into nude mice were composed of undifferentiated small cells typical of the EC cells seen in biopsies of human EC and of the stem cells found in transplantable human teratocarcinomas. Although human teratocarcinomas have been passaged by others in nude mice and in cheek pouches of cortisone-treated hamsters, only human teratocellular stem cell line NEC-8 has previously been reported to produce tumors in immunodeficient hosts (30).

All murine EC cells examined to date are characterized by the presence of the embryonic cell surface antigen, SSEA-1 (24), and by the lack of major histocompatibility antigens. A priori, there is no reason to assume that human EC cells must show a similar cell surface phenotype. However, in other cell lines (Tera-2 and SuSa) derived from human testicular germ cells, a large percentage of the cells lack HLA and \( \beta_2 \)-microglobulin and express a mouse embryonic antigen, F9 (11, 12), that evidently has been conserved during mammalian evolution. The F9 and SSEA-1 antigens show similar patterns of reactivity, and SSEA-1, like F9 antigen, is also detected on cells of the male germ line (11, 12), as indicated by analyses of cells in widely different passages.

Therefore, the expression of SSEA-1 by some 833K-E cells is consistent with an EC phenotype. However, this expression is not conclusive evidence that these are EC cells because SSEA-1, like F9 antigen, is also detected on cells of the male germ line and on some cells of the kidney and brain (24). The expression of HLA and \( \beta_2 \)-microglobulin on most, if not all, 833K-E cells provides a contrast with the murine teratocarcinoma stem cells. However, the level of expression is low and cannot be taken in isolation as evidence against the EC nature of 833K-E cells. We note that certain murine somatic cell hybrids have properties of pluripotent murine EC cells, although they express low levels of H-2 antigens (1). The aneuploid karyotype of 833K-E cells also contrasts with the near-diploid karyotype of many murine teratocarcinoma stem cells. In comparison, SuSa cells also have an aneuploid karyotype (11), as do the murine somatic cell hybrids (1). Thus, in some aspects (origin, morphology, and expression of SSEA-1), the 833K-E cells resemble other teratocarcinoma stem cells, and they also produce EC in nude mice. In other aspects, such as expression of major histocompatibility antigens, although weakly and in karyotype, they are dissimilar from mouse teratocarcinoma stem cells.

It is possible that 833K-E cultures contain more than one type of malignant cell and, perhaps, variable numbers of differentiated cells (which could lack SSEA-1 but express HLA and \( \beta_2 \)-microglobulin), with only the EC cells capable of producing tumors in nude mice. The cultures occasionally contain cells of different morphology, depending in large part on culture conditions, which might represent in vitro differentiation, other types of testicular tumor cells, or both. The immunofluorescence data also suggest heterogeneity of the cultures. The developmental relationships between these possible subpopulations, and the question of whether the stem cells express HLA and \( \beta_2 \)-microglobulin, will become clearer after studies of the clones derived from 833K-E and other human testicular tumor cell lines.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Phase-contrast photomicrograph of 833K-E cells in culture. × 300.

Fig. 2. Electron micrograph of 833K-E cells with desmosomes (thick arrows) and tonofibrils (thin arrows). × 60,000.

Fig. 3. Trypsin-Giemsa banding of chromosomes from 833K-E cells in passage 5 showing a hyperdiploid karyotype with a Y chromosome.

Fig. 4. Photomicrograph of a section of a tumor taken from a nude mouse inoculated with 833K-E cells. The tumor consisted of undifferentiated cells organized into convoluted sheets. H & E, × 240.
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