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 Caucassian 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° 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.

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

Antiserum. 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 reacts with an anti-clonal antibody, anti-SSEA-1, which recognizes a stage-specific mouse embryonic antigen and which reacts with undiluted trypsin:citrate.

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).

Cytochemistry. Chromosomal analyses were performed with 833K-E cells in passages 5 and 59 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 1**

<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.
of lymphocytes that were lysed by antibody, are: 1 - 0 to 10%; 2 = 11 to 25%;
that the cells are of human male origin, and, although the
able from the parent line. Chromosome analyses demonstrated
these same properties and are morphologically indistinguish
4 = 26 to 50%; 6 = 51 to 80%; 8 = 81 to 100%.

blood lymphocytes of known HLA type. The scores, representing the percentage
or anti-HLA-B12 (6) and of absorbed alloantisera were titered on peripheral
Although human teratocarcinomas have been passaged by

cal of the EC cells seen in biopsies of human EC and of the

karyotype of 833K-E cells also contrasts with the near-diploid
hybrids have properties of pluripotent murine EC cells, although

It is possible that 833K-E cultures contain more than one

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Human and mouse embryonal carcinoma cells in culture share an embryonic

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Chart 2. Cytotoxicity assays of unabsorbed alloantisera (C) and of sera after
incubation with 833K-E (∆), 833K-LC (□), or 1156Q-E (○) cells. C/C (□), complement control. The activities of unabsorbed alloantisera, anti-HLA-A2 (A) or anti-HLA-B12 (B) and of absorbed alloantisera were titered on peripheral
blood lymphocytes of known HLA type. The scores, representing the percentage
of lymphocytes that were lysed by antibody, are: 1 = 0 to 10%; 2 = 11 to 25%;
4 = 26 to 50%; 6 = 51 to 80%; 8 = 81 to 100%.

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.

Although human teratocarcinomas have been passaged by
others in nude mice and in cheek pouches of cortisone-treated
hamsters, only human testicular tumor 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 must show
a similar cell surface phenotype. However, in 2 other cell
lines (Tera-2 and SuSa) derived from human testicular germ
cancer, a large percentage of the cells lack HLA and β2-
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 reactivities, and SSEA-1, like F9, is detected on Tera-2 (24)
and SuSa cells.4

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 β2-microglobulin on most, if not all,
833K-E cells provides a contrast with the murine teratocarci-
noma 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 com-

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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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