UM-EC-1, a New Hypodiploid Human Cell Line Derived from a Poorly Differentiated Endometrial Cancer

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ABSTRACT

The University of Michigan endometrial carcinoma cell line UM-EC-1 was derived from a poorly differentiated endometrial adenocarcinoma of a 66-yr-old white female. Cell cultures were started using both tumor explants and a cell suspension obtained from collagenase-treated tumor tissue. The collagenase-derived cell suspension gave rise to monolayer cultures which grew rapidly from the outset. This subline of UM-EC-1 has now been subcultured more than 50 times. Cells derived from the tumor explants grew more slowly initially, but after a lag phase of 5 to 6 wk, this subline also exhibited rapid logarithmic growth and reached the same growth rate as that of the collagenase-treated cells. The explant subline has been subcultured more than 37 times. The doubling time of both sublines is 24 h under optimal growth conditions. The karyotype of both cell cultures is 43, XX, inv(1)(p32q24), −4, +der(8) t(8;12)(p23.1;q22), del(9)(q11), −13, −13, +t(13;13) (p13;p13), del(18)(q1), −19, −22, −23, +t(22;22)(p11:p11). The net result of the chromosome losses and rearrangements was monosomy 4, duplication 8p23.1→qter, deletion 9q11→qter, duplication 12q22→qter, deletion 18q and monosomy 19. The t(13;13) and the t(22;22) were dicentric by C-banding. Virtually all of the chromosome changes were stable in multiple passages except that there was mosaicism for chromosome 13. Some cells contained a single copy of 13 and others had t(13;13). The available evidence indicates the t(13;13) is an isochromosome. UM-EC-1 cells produced tumors histologically similar to the original tumor in male, female, and ovariectomized female athymic mice. UM-EC-1 cells express human class I histocompatibility antigens as assessed by binding of antibodies to nonpolymorphic HLA and β2-microglobulin antigens. Blood group antigens A and H were absent although the patient is blood type A and these antigens are normally expressed in endometrial glands. A rearrangement involving the region of chromosome nine that carries the ABH locus may be related to the absence of blood group antigen expression by these cells. The E7 membrane antigen, the locus for which resides on the short arm of chromosome 11, was expressed strongly which is consistent with the presence of two intact copies of chromosome 11 in these cells.

INTRODUCTION

The UM-EC-1 cell line described in this paper was established from tumor tissue of a patient with advanced, poorly differentiated endometrial carcinoma. In the course of laboratory studies, this cell line was found to be very sensitive to tamoxifen but resistant to the progesterone analogue medroxyprogesterone acetate. The donor of UM-EC-1 had failed to experience an objective clinical response (1). These findings provide a single example of how in vitro cultures can be used to study the biological behavior of cancer cells.

Based on this clinical situation and the laboratory findings, the patient was subsequently treated with tamoxifen and experienced an objective clinical response (1). These findings provide a single example of how in vitro cultures can be used to study the biological behavior of cancer cells.

The development and analysis of new cell lines at early passage are important to provide investigators with cells whose characteristics are close to the primary malignancy. Twelve endometrial carcinoma cell lines were reviewed by Richardson et al. (2) when they reported the development of the KLE line in 1984. Since then three additional endometrial carcinoma cell lines have been reported (3–5). Some of these cell lines have been used to study the effects of steroid hormone action at the cellular level, a clear knowledge of which is important for therapy of endometrial carcinoma and may provide clues to the etiology of this cancer type as well (5–11). Chromosome analysis of endometrial carcinoma has typically revealed near diploidy (2, 12–15), although Slot et al. (12) found polyplody to be associated with poorly differentiated tumors. Near tetraploid cells have been found to represent a minor population in several cell lines (14, 15). Among cell lines studied cytogenetically using banding techniques, one diploid (14), one trisomy 8 (15), and two karyotypically complex endometrial adenocarcinoma cell lines have been described (2, 16).

In this report the isolation and characterization of UM-EC-1, a hypodiploid cell line derived from the primary site of a poorly differentiated adenocarcinoma of the endometrium, is described including (a) the histopathology of the original tumor and tumors produced by UM-EC-1 cells in nude mice; (b) the growth characteristics of the cells in culture and in nude mice; (c) a complete cytogenetic analysis of the cultured cells; and (d) an assessment of the cell surface antigens expressed with reference to the chromosome content, where applicable.

MATERIALS AND METHODS

Clinical History. A 66-yr-old gravida 2, para 2 white female presented with a history of intermittent postmenopausal bleeding in 1986. A curettage was performed and she was found to have poorly differentiated endometrial adenocarcinoma Stage 1 B Grade 3. She underwent total abdominal hysterectomy, bilateral salpingooophorectomy, paraaortic node biopsy, and aspiration of a 2-cm cystic mass in the liver. Invasion through one-half of the myometrium as well as involvement of the cervix and paraaortic lymph nodes was found.

Culture Techniques. A specimen from the uterine tumor was cultured using two techniques. One part was minced with small scissors and incubated with 0.1% collagenase (Cooper Biomedical) for 30 min at 37°C. The collagenase was neutralized by dilution with DMEM supplemented with 15% fetal bovine serum, 1% (v/v) nonessential amino acids, 2 mM l-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin. The suspension was centrifuged at 700 × g for 10 min, the sediment was resuspended in DMEM, and the tissue fragments and large cell clusters were allowed to settle for 45–60 sec. The

1 Supported by United States Public Health Service Grant CA 28564 from the National Cancer Institute and a grant from the Cancer Society of Finland.
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4 The abbreviation used is: (c)DMEM, (complete) Dulbecco's minimal essential culture medium.
supernatant of single cells and small cell clusters was transferred to 25-
cm² culture flasks in growth medium consisting of a 1:1 mixture of
cDMEM and Ham's F-12 with 60 IU/liter insulin.

The second part of the tissue was cut into approximately 1-mm³
fragments which were placed in 25-cm² culture flasks. The explants
were allowed to adhere to the surface for 10 min before addition of 2
ml of growth medium. The cultures were incubated at 37°C in a
humidified atmosphere containing 5% CO₂ and 95% air for 18 h after
which the flasks were tightly closed. The flasks containing explants
were incubated undisturbed for 7 days after which the cultures were
observed and fed weekly. Cells from confluent flasks were harvested by
trypsin/EDTA in Pock's saline A (315 μ/ml trypsin, 0.2% EDTA). Cells
were tested for mycoplasma at passage 6 and were found to be
free of contamination.

Cell Growth Experiments. Cells in either subconfluent logarithmi-
cally growing cultures or in hyperconfluent cultures were harvested
with trypsin/EDTA, counted, adjusted to the appropriate cell density
in cDMEM or in DMEM containing 5% dextran charcoal-treated fetal
bovine serum (D₃) and distributed in equal amounts to the wells of
multiple 6-well plates (Costar, Cambridge, MA). The plates were incu-
bated at 37°C in a water vapor-saturated atmosphere of room air
containing 5% CO₂. The medium was replaced daily. On day 1 and on
odd numbered days thereafter the cells from three replicate wells from
each group were carefully trypsinized and counted using a hemocytom-
eter. Viable cells were determined by trypsin blue exclusion. Optimal
growth rate was assessed using cells harvested from subconfluent cul-
tures in logarithmic growth whereas the effects of high cell density and
the plateau phase of cell growth on subsequent growth rate were
assessed using cells from hyperconfluent cultures.

Heterotransplantation. In five different experiments s.c. tumors were
produced in athymic (nu/nu CD1) nude mice inoculated with UM-EC-1
cells. The number of inoculated cells varied from 10⁶ to 2.5 × 10⁷
cells per flank. Cells were trypsinized, washed, counted, and injected
s.c. into the right and left flanks of normal female, normal male, and
ovariectomized female mice. At weekly intervals the tumors were mea-
sured in 3 dimensions using adjustable calipers (17). After 2 mo the
mice were killed by cervical dislocation and the tumors were removed
and studied histologically.

Cell Surface Antigen Phenotype. Cells growing in monolayer cultures
in 96-well plates (Costar) were tested for expression of a variety of cell
surface antigens using hemadsorption assays (18, 19). Rabbit anti-
serum to human β-2-microglobulin, mouse monoclonal antibodies to
nonpolymorphic class I HLA antigens, to blood group antigens A, B, and
H (20) and to the ubiquitous cell surface antigen E7 (18, 21, 22), as
well as human tumor specific cell group typing sera were used as serological
reagents. Rabbit anti-human β-2-microglobulin serum and DAKO anti-
A and anti-B monoclonal antibodies were purchased from Accurate
Chemical and Scientific Corporation of Westbury, NY. Monoclonal
antibody W6/32 to nonpolymorphic HLA determinants (23) was gen-
erosly provided by Dr. Walter Bodmer. Antibodies G10 and E7 were
developed in our laboratory (18).

UM-EC-1, UM-SCC-11A, and UM-SCC-38 cells used for absorp-
tion were harvested from tissue culture flasks using trypsin/EDTA. All
cells used for absorption were washed with phosphate-buffered saline
prior to absorption. Dilutions of blood group typing sera (Ortho Di-
gnostics, Raritan, NJ) two tubes below the endpoint of agglutination
were harvested from tissue culture flasks using trypsin (Difco 1:250) and Giemsa (Harleco). Selected slides were Q-
banded, C-banded, or stained by the Ag-NOR technique. To assess the
presence and frequency of nuclear projections, cultures grown on glass
slides (LabTek, Miles, IN) were treated with hypotonic 0.075 M KCl,
fixed, and stained in situ.

A primary objective of the chromosome analysis was to identify one
or more consensus karyotypes. A large number of metaphase cells were
compared to identify the consistent chromosome makeup of the cell
line. The constant or most consistent karyotype observed comprises the
consensus karyotype of the in vitro stem line. Through this approach
changes present only in individual cells representing random loss, gain,
or rearrangement can be ignored, and minor side lines can be identified.

RESULTS

Histology and Cell Culture. The histological appearance of the
patient's tumor as well as that of a representative tumor
formed in nude mice by inoculation of cultured UM-EC-1 cells
are shown in Fig. 1. The histology of the patient's primary
tumor is consistent with the diagnosis of poorly differentiated
adenocarcinoma (Fig. 1, A and B). Cytology of the fluid aspirated
from the liver cyst also revealed cells with characteristics
consistent with metastatic endometrial adenocarcinoma (not
shown). The nude mouse tumor recapitulates the histology of
the patient's tumor (Fig. 1, C and D). In both cases there were
extensive areas of necrosis.

In cultures of UM-EC-1 initially obtained by treatment of
tumor fragments with collagenase, proliferation of cells was
observed within 2 days. The growth medium was changed, and
after 9 to 14 days the flasks were confluent. The cells in these
cultures had a characteristic epithelioid morphology (Fig. 2).
Cells from confluent flasks were trypsinized and passaged to
new flasks with a 1:2 dilution. No fibroblasts were observed in
these flasks.

In cultures started with tumor explants, dividing cells were
seen after 7 days in culture, at which time the culture medium
was changed. Thereafter the medium was changed once a week.
Around 5 to 6 wk, these flasks became confluent and the first
passages were done. Although some fibroblasts were observed
in the primary explant flasks, they were overgrown by tumor
cells in secondary passage so that there was no need for selective
tryptinization to isolate the tumor cells. The tumor cells growing
out from tumor explants appeared to have a greater tendency
to grow in multilayer cultures (Fig. 2A) than did cells from the
collagenase-derived suspension, which tended to grow as
monolayer cultures.

In Vitro Growth Rate. Growth curves were performed on
cells of both the explant-derived and collagenase-derived
cultures. Results of 3 representative experiments are presented in
Fig. 3. There were no significant differences in growth rate
among the explant- and collagenase-derived cultures or cultures
fed with cDMEM or D₃ when assessed in multiple passages.
This was in spite of the observation that the cultures established
from the cell suspension obtained by collagenase treatment of
the tumor grew very rapidly immediately after being placed in
culture, whereas the cultures from the explants took several
weeks to become established. However, the growth rate of cells
taken from hyperconfluent cultures was slower than that of
cells taken from log-phase cultures. This effect was consistent
both in the explant-derived and collagenase-derived cultures in
all passages that were tested. As shown in Fig. 3, the cell
population doubled 8 times in 11.3 days (from 3 × 10⁶ cells to
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Fig. 1. Histological appearance of tissue from the patient's primary tumor (A and B) and of a s.c. tumor mass grown in a female nude mouse inoculated with 2 × 10⁷ passage 7 UM-EC-1 cells (C and D). A, low power illustrates poorly differentiated endometrial adenocarcinoma. H & E, ×80. B, higher power photomicrograph of a different area of the same tumor section illustrates marked pleomorphism, abnormal mitotic activity, and necrosis. H & E, ×400. C, photomicrograph taken at the margin between the mouse skin (left) and the tumor tissue (right) demonstrates marked pleomorphism characterizing the anaplastic appearance of the tumor. H & E, ×280. D, photomicrograph of a different area of the same UM-EC-1 tumor in the same nude mouse demonstrates the marked pleomorphism, abnormal mitotic activity, and necrosis which were characteristic of the primary tumor specimen. H & E, ×200.

about 7.7 × 10⁶ cells) when cells from cultures in logarithmic growth were tested. The average doubling time was 33 h, and a maximal doubling time of 24 h was calculated at midlogarithmic growth (from 6 × 10⁶ cells to 2.4 × 10⁷ cells per well). In contrast, the time necessary for cells from confluent cultures to undergo 8 population doublings was 17 days. In these cultures the average doubling time was 51 h and at maximal growth rate was 48 h (calculated at midlogarithmic growth from 6 × 10⁶ cells to 2.4 × 10⁷ cells per well). The saturation density was the same in both cases: 1.2 × 10⁷ cells per 35-mm well, which corresponds to 1.25 × 10⁶ cells per cm².

After 23 to 26 days of culture in the plastic wells, clusters of cells were found to detach from the substrate in spite of daily feeding. Similarly, at confluence UM-EC-1 cells can be shaken off the plastic by gently tapping the vessel and at any time during culture the cells can be readily trypsinized in 1 or 2 min at room temperature.

Heterotransplantation. Cells from both UM-EC-1 subpopulations produced tumors in nude mice. In initial experiments, there was a sex difference favoring growth of tumors in male mice (7 of 9) compared to female mice (5 of 9). Therefore additional tests were performed with female, male, and ovariectomized female mice in subsequent experiments. No clear gender effect was seen in those experiments and overall tumors developed in 9 of 14 female mice, 7 of 8 ovariectomized female mice, and 12 of 14 male mice.

Cell Membrane Antigen Phenotype. UM-EC-1 cells express class I HLA determinants as detected by rabbit antisera to human β-2-microglobulin and by murine monoclonal antibodies to nonpolymorphic HLA heavy chain determinants (Fig. 4). Since normal endometrial glandular cells from multiple donors were found to express blood group antigens as determined by immunoperoxidase assays on frozen tissue sections (data not shown), we expected that these antigens should also be present on UM-EC-1. The donor of UM-EC-1 is blood group A. However, blood group antigens were not detected on UM-EC-1 cells by direct hemadsorption assays with monoclonal antibodies to blood group A or B. Since tumor cells from blood group positive tissues often exhibit enhanced expression of the H type 2 precursor antigen we also tested UM-EC-1 cells for expression of the H type 2 antigen using the G10 monoclonal antibody (20). No binding of G10 could be detected. Similarly, absorption of blood group typing antibodies with UM-EC-1 cells also failed to demonstrate the presence of blood group A or B on the tumor cells, whereas red blood cells and squamous carcinoma cells from blood group A donors did absorb blood group A antibodies (Table 1).

The monoclonal antibody E7 reacted strongly with UM-EC-1 cells. This antibody defines a ubiquitous cell surface antigen whose expression is controlled by a gene that maps to the p13 region of chromosome 11 (21, 22). The level of reactivity of UM-EC-1 with E7 antibody (Fig. 4) was the same as that of E7.
with cultured normal diploid fibroblasts and normal endometrial stromal cells (data not shown).

Chromosome Analysis. The karyotype of the normal lymphocytes and of the Epstein-Barr virus transformed lymphoblasts from the UM-EC-1 donor was 46, XX with no visible abnormalities.

Karyotype of Primary Explant Culture. The initial chromosome analysis was performed on 19 G-banded metaphase cells from a primary culture flask initiated with tumor explants. The metaphase cells from this culture contained from 12 to 45 chromosomes, with no clear modal number.

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<th>Chromosome count</th>
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Most of the hypodiploid cells were broken, resulting in random hypodiploidy. Two of the metaphase cells were hypotetraploid, indicating that these two cells were most likely broken as well. Most of the variation in chromosome counts in this harvest from the primary culture flask appeared to represent preparational artifact. The karyotype of the cell with 44 chromosomes is shown in Fig. 5. This cell is representative of the consensus karyotype of the UM-EC-1 (see below) except that it contains a single chromosome 13 rather than the more common finding of t(13;13).

Karyotype of Explant Culture Passage 2. Sixty-five G-banded metaphase cells from passage 2 of a companion explant culture were counted and 13 metaphases were completely analyzed. Metaphase cells from passage 2 contained from 29 to 82 chromosomes, with most cells having 42 to 45 chromosomes.

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The karyotypes of these cells were very similar to each other with the differences among cells apparently due to random loss and gain of individual chromosomes. Based on the 13 cells that were fully analyzed a consensus karyotype was derived. The consensus is as follows: 43, XX, inv(1)(p32q42), -4, +der(8)(p13;13)(p12.1;q22), del(9)(q11), -13, -13, +t(13;13) (p13;p13), del(18)(q), -19, -22, -22, +t(22;22)(p11;p11) (Table 2). The consensus karyotype is the same as that shown in Fig. 5 except that the cells from the primary flask had monosomy 13 (Fig. 5) whereas all 13 of the analyzed cells from this culture had a dicentric chromosome 13 (Fig. 6D). The rearrangements consistently observed in passage 2 are illustrated in Fig. 6. The net result of the chromosome losses and rearrangements was monosomy 4, duplication 8p23.1 -> qter, deletion 9q11 -> qter, duplication 12q2 -> qter, deletion 18q, and monosomy 19. The inversion of chromosome 1 appeared to be balanced. The t(13;13) and the t(22;22) could represent either isochromosomes or Robertsonian translocation of the homologues.

Karyotypic Analysis of Other Passages. Cells were also karyotyped from passages 4 and 17 of explant-derived cultures and passages 2, 12, and 43 of collagenase-derived cultures. Among the 119 cells scored from the primary culture and from cultures at passages 2 and 4 of explant derivation and passage 2 of collagenase derivation, the following karyotypic features were highly consistent: monosomy 4 and 19; +der(8); del(9); del(18); and t(22;22). Examples of these rearrangements are provided in Figs. 5 and 6. The chromosome 13 variations in 269 cells are detailed in Table 3.

Analysis of the Ring Marker. A small marker chromosome was found in 64 of 119 cells scored in three different cultures (Table 3). This is a ring chromosome which we speculate was derived from the centromeric portion of chromosome 4 since 4 and 19 were the only chromosomes lost and since the C-banded portion of the ring corresponded to that of chromosome 4 better than to that of chromosome 19 (Fig. 6G).

Analysis of Chromosomes 13 and 22. The t(13;13) and the t(22;22) were dicentric by C-banding. The t(22;22) was Ag-NOR negative. The t(13;13) had a large pale staining gap between the centromeres with a central light grey band that probably represent band 13pl2 (Fig. 6D). With the Ag-NOR technique, about two-thirds of the region stained positively for nucleolar activity, while the remaining one-third was not stained (Fig. 6D).

Whereas the t(22;22) was consistently present in all cultures, there was mosaicism involving chromosome 13 (Table 3). In hypodiploid cells from the primary culture only monosomy 13 was found. Passages 2 to 4 of the explant culture as well as all passages of the collagenase-derived cultures had mosaicism with either monosomy 13 or t(13;13). Cells in explant culture pas-
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Fig. 5. Karyotype from the primary culture of endometrial carcinoma UM-EC-1. The consensus karyotype is detailed in the text and Table 1. In this cell there is monosomy 4, 13, and 19. There is a pericentric inversion of chromosome 1, deletion of chromosome 9q, deletion of 18q, and isochromosome or Robertsonian fusion 22q. There is an extra chromosome 8 with about half of 12q attached to its short arm, resulting in duplication of most of 8 and of the 12q segment. The ring chromosome, observed in about half of cells, appears to be a ring 4 (see Fig. 6 and text).

Table 2 Karyotypes of 13 fully analyzed cells from a UM-EC-1 explant culture at passage 2

The most commonly observed finding for each chromosome was taken as the consensus karyotype. Cells 4 and 10 on this table express the consensus karyotype. Cell number 12 contained the consensus karyotype plus the ring (47) marker. Except for mosaicism for presence or absence of the ring chromosome, all of the differences among cells were considered artifactual. Whereas most cells had two 8s plus the derived 8, two cells, 2 and 13, had only one normal 8 plus the derived 8. Cell 6 had two normal 8s and two derived 8s. Cell 1 had trisomy 5 and trisomy 16. One cell each had a t(1;7) involving the usually normal 1 chromosome (cell 3), nullisomy 4 (cell 5), duplication 7pter-p22 (cell 7), monosomy 6 (cell 11), monosomy 9 (cell 8), trisomy 11 (cell 9), and monosomy 12 (cell 11). The consensus karyotype is given in column two. How each individual cell (#1-13) differed from the consensus is shown in the right side of the table.

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</tbody>
</table>

Markers

*a t(1;7)(p36.3;p15)  
b dup(7)(p15;p22)  
c +der(8)  
d monosomy 9  
e +tiny ring chromosome  
f ok = 2 normal chromosomes

sage 17 lacked the t(13;13) and had either one or two copies of chromosome 13 in the diploid cells. Since no cells were found in the primary culture with two normal 13s, the t(13;13) may represent a true isochromosome rather than a translocation between homologous 13s.

Nuclear Projections and Dicentric Chromosomes 13 and 22. Interphase nuclear projections (blebs) have been reported to occur in cell lines carrying a dicentric chromosome (26). Since UM-EC-1 cells have dicentric chromosomes we evaluated whether such projections were present. Nuclear projections were observed in interphase cells from all passages of UM-EC-1 cells examined. Seven of 100 interphase cells scored in UM-EC-1 collagenase-derived cultures (passages 2–4) had nuclear projections Fig. 7 whereas nuclear projections were never observed in control cells from a normal male fibroblast cell line (500 cells scored). This is evidence for functional activity of
Fig. 6. Partial karyotypes from endometrial carcinoma UM-EC-1. A, pairs of chromosome 1 from three cells illustrate a pericentric inversion (right) with breakpoints at 1p32 and 1q42. B, der(8) from two cells is shown in the center between pairs 8 and 12 from the same cells to illustrate the origin of the der(8) from a t(8;12) (p23.1;q22). The number 12 pair is shown upside down to compare banding patterns with the derived chromosome. C, two 9 pairs show that most of 9q is deleted from one homologue. D, isochromosome 13 or Robertsonian fusion 13;13 from a G-banded cell (left) illustrates the large amount of short arm material remaining. There are two C-band positive blocks on this chromosome (next two examples), and the two examples at right illustrate that much of the material between the centromeres is Ag-NOR positive. E, two 18 pairs show the deletion of 18q. This could be either deletion 18q12.3 —> qter or an interstitial deletion of 18q11.2 —> q21.3. F, isochromosome 22 or Robertsonian fusion 22;22 is shown in two G-banded examples. G, three G-banded examples of the tiny ring are shown on the right. At the right examples of chromosome 4, the ring, and chromosome 19 are shown after C-banding to compare the size of the centromeric heterochromatin.

both centromeres on either t(13;13), t(22;22), or both.

Since the two centromeres on chromosome t(13;13) were separated by a wide gap it was possible to determine the number of primary constrictions on this chromosome. In the primary culture the t(13;13) had 2 primary constrictions in 6 of 13 cells. Likewise, in the passaged cultures the t(13;13) had 2 primary constrictions in about one-half of the cells.

To determine whether the t(13;13) or t(22;22) are isochromosomes we examined the patient's normal lymphocytes for Q-band variance on the chromosome 13 and 22 paracentromeric region. Both of the normal lymphocyte 22cs were pale to medium in fluorescence and for this reason were noninformative. However, the normal lymphocyte 13cs differed in fluorescence intensity. One was pale to medium and the other was medium to intense in fluorescence. Both of the 13c bands in the t(13;13) of the UM-EC-1 cultures exhibited pale to medium fluorescent staining as did the 13c band of the solitary chromosome in UM-EC-1 cells with monosomy 13. This result suggests that the t(13;13) represents an isochromosome rather than a Robertsonian translocation. The foregoing observations are consistent with monosomy as the original karyotypic event affecting chromosome 13. Subsequent events then may have led to isochromosome formation or (in all except the passage 17 explant culture) nondisjunction leading to a cell population with two copies of the single homologue. The possible origin of the isochromosome 13 is depicted diagrammatically in Fig. 8 and described in the legend.

**DISCUSSION**

UM-EC-1 has characteristics typical of malignant endometrial cells, including aneuploidy, continuous growth in tissue culture, and the capacity to produce in athymic mice poorly differentiated tumors histologically similar to the original tumor. In vitro the tumor cells tend to pile up or grow in 3 dimensions. UM-EC-1 cells grow to a density of more than one million cells/cm$^2$, which is the highest density we have observed with human tumor cells. The cells are also poorly adherent in culture, especially at high cell density. This is consistent with the loose histological appearance of the cells in tissue sections.

The karyotype of UM-EC-1 is well defined and was remarkably stable over many passages. It is also consistent with reports in the literature that in general describe a limited range of chromosome abnormalities in endometrial carcinomas. Trent and Davis (13) reported "D group" abnormalities in two of three adenocarcinomas, including a t(13;15) and a t(1;15). Working with cytogenetic preparations directly from tumor tissue, Slot (12) found that 17 of 20 well-differentiated endometrial carcinomas were hypodiploid and that 7 of 9 poorly differentiated carcinomas were polyploid. In other studies of short-term cultures or direct preparations of endometrial carcinomas several types of chromosomal abnormalities were found including duplication or triplication of the long arm of chromosome 1 (27); chromosome 8, 12, and X abnormalities (28); duplication of 1q and deletion of 1p (29); trisomy 10 and +1q (30). In several cases polyploid cells were also observed. Using flow cytometry Iverson (31) found that 14 of 52 endometrial carcinomas had more than one identifiable cell popu-

**Table 3. Summary of distribution of chromosome 13 and the marker ring in UM-EC-1 cultures**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Passage</th>
<th>No. of cells</th>
<th>Diploid</th>
<th>Tetraploid</th>
<th>Ring marker (t(13))</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>13</td>
<td>iso 13</td>
<td>2 13s</td>
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<tr>
<td>Explant-derived</td>
<td>Primary</td>
<td>19</td>
<td>17</td>
<td>6</td>
<td>37</td>
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<tr>
<td></td>
<td>2-4</td>
<td>50</td>
<td>6</td>
<td>37</td>
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</tr>
<tr>
<td></td>
<td>17</td>
<td>50</td>
<td>6</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Collagenase-derived</td>
<td>2</td>
<td>50</td>
<td>6</td>
<td>37</td>
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<td></td>
<td>12</td>
<td>50</td>
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<td>37</td>
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<tr>
<td></td>
<td>43</td>
<td>50</td>
<td>6</td>
<td>37</td>
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<td>Total</td>
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<td>269</td>
<td>85</td>
<td>114</td>
<td>12</td>
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</tbody>
</table>

* N/C—not counted.

* Three cells with other 13 rearrangements were also observed.

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A new endometrial cancer cell line (UM-EC-1) was near diploid with relatively few chromosomal changes, all of which could be defined with confidence. Chromosome 1 rearrangements are frequent in endometrial carcinomas (13, 27, 29, 30). This is consistent with our finding of a pericentric inversion of chromosome 1 with breakpoints at 1p32 and 1q42, although in UM-EC-1 the rearrangement appears to be balanced. That a v-myc homologue has been identified in lung cancer at the 1p32 locus is a finding that may in the future be found to have significance if there is an alteration in expression or structure of the myc protein in this cell line.

UM-EC-1 also contains a consistent rearrangement involving chromosomes 8 and 12. In the simplest analysis, this rearrangement results in duplication chromosome 8p23.1→qter and duplication chromosome 12q22→qter. This might be significant if increased expression of oncogenes such as c-myc and c-mos on the long arm of chromosome 8 (32, 33) or the cellular homologue of v-int-1 on chromosome 12 or the insulin-like growth factor (locus at 12q22→q24) can be demonstrated in UM-EC-1. Trisomy 8 was consistent among all three poorly differentiated endometrial carcinoma cell lines that have been karyotyped with banding: RL95-2 (14), KLE (2), and UM-EC-1. The other alterations found in UM-EC-1 were monosomy 4 and 19 and deletions 9q11→qter and 18q. Loss of 19 was also described in KLE cells (2).

In this investigation we studied the expression of cell surface antigens whose chromosome loci have been mapped. Expression of blood group A and H antigens could not be demonstrated in UM-EC-1 cells. Loss of mature blood group antigen expression is common in squamous carcinomas (34). However, failure to express the precursor is rare. The mechanism which controls blood group expression is not known, nor is the mechanism by which tumor cells fail to express blood group. However, one possibility we have considered is that a defect involving deletion of chromosome 9q like that observed in UM-EC-1 cells could result in the loss of expression of the ABH genes which have been mapped to 9q34.
Jones et al. (21) demonstrated that the E7 antigen was associated with a locus on the short arm of chromosome 11. Expression of the E7 antigen was strong in UM-EC-1 which is consistent with the presence of two apparently normal copies of chromosome 11. Human class I HLA antigens were expressed at levels comparable to other cell types. This is also consistent with intact chromosomes 6 (HLA heavy chain) and 15 (β-2-microglobulin).

The translocations involving chromosomes 13 and 22 could represent either simple Robertsonian fusions between the homologues or could represent the isochromosomes (genetically identical arms) with loss of the homologue via nondisjunction. The distinction between these two possibilities is important because a nondisjunction-isochromosome mechanism would result in homozygosity of all of the alleles on the chromosome and would permit expression of recessive genes or mutations. For example, the Rb-1 allele on chromosome 13 that has been described in retinoblastoma (35, 36) appears to function as a recessive oncogene. The monosomy 13 observed in the primary culture of UM-EC-1 is circumstantial evidence that a primary nondisjunctional event occurred which in some cells was followed by isochromosome formation. Q-banding of the 13s in the patient's normal cells and homogenous Q-banding of the dicentric t(13;13) indicate that the t(13;13) is more likely an isochromosome than a Robertsonian fusion. (Q-banding analysis of chromosome 22 was not informative.) Isochromosome formation might have a selective advantage over monosomy because doubling the dose of “housekeeping” genes on these chromosomes would return the cell to normal balance with respect to these genes. Dutrillaux and Couturier (37) hypothesized that such chromosome changes in endometrial adenocarcinoma may confer advantage to cells which have extra copies of segments that carry housekeeping genes. The isochromosome 13 tends to predominate in most of the later passages with the exception of the subline represented by explant P17. This may indicate that growth advantage is associated with the isochromosome. We are presently isolating clones from UM-EC-1 to compare the monosomic cells with those containing the t(13;13). Molecular genetic analysis now underway may provide evidence of heterozygosity or homozygosity of loci on 22, confirm homozygosity on chromosome 13, and identify submicroscopic deletions on 13 or 22.

All of the UM-EC-1 cultures were similar in having about 15% near tetraploid cells. Tetraploidy was not the result of colcemid-induced endoreduplication (C-mitosis), since these cells were observed in metaphase preparations not treated with colcemid (7 of 50 cells scored from explant passages 2-4). There may be a stable subpopulation of tetraploid cells, but it is perhaps more likely that they arise from abnormal mitoses of the near diploid cells since in each subline the proportion of chromosome 13 variations was similar in both the near tetraploid and the near diploid cell populations (Table 3).

UM-EC-1 is a highly tumorigenic cell line with well-defined phenotypic and cytogenetic markers. Several chromosome abnormalities have been found which are highly stable and consistent in the majority of cells from multiple passages. Furthermore, several of the chromosome breakpoints have occurred near or at the location of known oncogenes. These observations suggest that these are not random changes but rather may represent the karyotypic events that initially led to the transformed behavior of these cells. Changes involving chromosomes 1, 8, 12, 14, and 19 that are similar to those found in UM-EC-1 have been reported by several other authors (2, 13, 15, 16, 27, 28, 30) and may prove to be consistent markers of endometrial carcinoma.

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UM-EC-1, a New Hypodiploid Human Cell Line Derived from a Poorly Differentiated Endometrial Cancer

Seija E. Grenman, Daniel L. Van Dyke, Maria J. Worsham, et al.


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