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Establishment and Characterization of UM-EC-2, a Tamoxifen-Sensitive, Estrogen Receptor-Negative Human Endometrial Carcinoma Cell Line[1,2]

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UM-EC-2 was established from a patient with poorly differentiated stage IB endometrial carcinoma. This cell line produces tumors in nude mice that have the same histological features as the patient's tumor. UM-EC-2 cells express β_2-microglobulin, the epidermal growth factor receptor (EGF), and the H blood group antigen. This membrane antigen phenotype is consistent with cells of human endometrial origin. The karyotype of UM-EC-2 is fairly complex, with rearrangements affecting all chromosomes except 3, 10, 14, 19, and 20. There were two populations of cells, a hyperdiploid population with a modal number of 53–55 and a hypertetraploid population with a modal number of 109. A postulated sequence of events before and after tetraploidization is suggested based on the number of copies of individual chromosomes and rearrangements. Comparison of the UM-EC-2 karyotype to that of UM-EC-1 (a previously described line from a different patient with endometrial carcinoma) revealed that the two lines share eight very similar chromosome changes, which include loss of most of chromosome 4, breakpoints affecting proximal bands on 8p, loss of most of 9q, a breakpoint at 12q22, loss of 13q, breakpoints in proximal bands on 18q, and a breakpoint at 22p11. These changes may represent nonrandom chromosome abnormalities in poorly differentiated endometrial cancer. Estrogen (ER) and progesterone (PgR) receptors were not detected in either the primary tumor or the cell line. Nevertheless, UM-EC-2 cells were very sensitive to growth inhibition by tamoxifen (TAM) *in vitro*. One micromolar TAM caused 50% inhibition of cell growth, 2.5 μM caused cytostasis, and 5 μM TAM was cytotoxic, killing all cells after 5–7 days of exposure to the drug. Paradoxically, 100 nM estradiol (E_2) caused a moderate increase in the growth of the cells but it did not prevent or reverse growth inhibitory effects of TAM. These findings support the concept that in some tumors TAM causes growth inhibition by an ER-independent mechanism. UM-EC-2 cells were also sensitive to growth regulation by EGF. Thus, these cells provide a new *in vitro* model of human endometrial cancer in which the roles of both TAM and EGF as growth regulatory substances can be investigated. © 1990 Academic Press, Inc.

INTRODUCTION

The standard therapy for endometrial cancer is surgery but for residual disease after surgery or for the treatment of recurrent and advanced endometrial carcinoma, hormonal therapy is usually employed. Objective responses have been achieved with progestins in 30–35% of patients [1,2]; however, this treatment has not improved the 5-year survival of patients with advanced or recurrent disease [3,4]. This response rate is not unexpected since many advanced tumors are poorly differentiated and fail to express steroid hormone receptors [5–7]. Tamoxifen (TAM) has also been used in treatments of this group of patients but often after failure with progestin therapy; nevertheless, a response rate of 20% has been obtained [8–14]. In our laboratory we have been investigating the growth regulatory effects of TAM on endometrial carcinomas *in vitro*. In experiments using four estrogen re-

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ceptor (ER)-negative, progesterone receptor (PgR)-negative, progestin-resistant cell lines derived from moderately or poorly differentiated endometrial carcinomas, we found that all four exhibited dose-dependent growth inhibition in response to TAM treatment [15]. We have now established and characterized a new ER-negative endometrial carcinoma cell line (UM-EC-2) from a patient with advanced poorly differentiated endometrial carcinoma and used this line to expand on our prior observations. In this report the characteristics of this line and the effects of TAM, estradiol (E_2), and epidermal growth factor (EGF) on its *in vitro* growth are described.

MATERIALS AND METHODS

Clinical History

The donor of the UM-EC-2 cell line was a 53-year-old gravida 3, para 3, white female with a prior medical history of lymphoma treated by radiation and chemotherapy. Ten years after that treatment, she was referred to the University of Michigan Medical Center for treatment of stage IB, grade 3 endometrial carcinoma. At the time of surgery she was found to have an extensive endometrial carcinoma invading the full thickness of the cervix and myometrium, with tumor eroding through the anterior wall and the left cornu of the uterus. Total abdominal hysterectomy, bilateral salpingo-oophorectomy, and partial cystectomy were performed. Postoperatively the patient was treated with medroxyprogesterone acetate (MPA) 160 mg/day. However, within 3 months, tumor progression was obvious, with metastatic spread to the lungs and bilateral ureteral obstruction. At this time treatment with doxorubicin was instituted. The patient failed to respond to this therapy and died of her disease soon after.

Cell Culture and Serologic Reagents

Tumor cells were cultured using standard techniques [16]. A portion of the tumor was transported to the laboratory where it was washed in triple-antibiotic solution consisting of Puck's saline A containing penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (40 μg/ml). The tissue was then cut into small fragments and placed into plastic flasks. The fragments were allowed to adhere for several minutes and were then covered with culture medium consisting of Dulbecco's modified Eagle's medium supplemented with 1% (v/v) nonessential amino acids, 20 m*M* L-glutamine, 15% fetal bovine serum (FBS), penicillin (100 μl), and streptomycin (100 μg/ml) (cDMEM). Fibroblasts were removed by brief treatments with trypsin and EDTA (315 U/ml trypsin activity and 0.2 *M* EDTA in Puck's saline A) as needed [16]. When the tumor cells became confluent in the culture flasks, the cells were subcultured by detachment with trypsin and EDTA. Cultures of UM-EC-2 were tested by standard microbiological tests and were found to be free of mycoplasma contamination. Cultures at several early passages were frozen in liquid nitrogen. Cells were also grown for inoculation into athymic mice (Nu/Nu CD1) (Charles River). Logarithmically growing tumor cells in passage 7 were harvested from 75-cm^2 flasks with trypsin–EDTA and 1×10^7 to 5×10^7 cells were inoculated under the skin on the flanks of two male and two female mice. Mice were examined at weekly intervals, and when the tumors reached a volume of approximately 0.5–1 cm^3 the mice were killed by cervical dislocation and the tumors were removed for histological examination.

Antibody reagents used to characterize the cell surface antigens included rabbit antiserum to human β_2-microglobulin and monoclonal antibodies to blood groups A and B (Accurate Chemical and Scientific Corp., Westbury, NY), human blood group typing antisera (Ortho Diagnostics, Raritan, NJ), and monoclonal antibodies UM-A9 and UM-G10 raised in our laboratory [17–20]. Autoantibodies to the squamous cell-specific pemphigus and pemphigoid antigens were used as specificity controls [16]. EGFR1 monoclonal antibody to the external domain of the EGF receptor [21] was purchased from Amersham, Skokie, Illinois. UM-EC-2 cells growing in monolayer cultures in 96-well plates were tested for antigen expression using protein A and anti-immunoglobulin hemadsorption assays as described previously [22].

Cytogenetic Analysis

UM-EC-2 cells in passage 1, passage 2, and passage 4 were harvested and analyzed as described previously [22,23]. Eighteen cells in passage 1 and fifteen cells in passage 2 were fully analyzed. In addition, four passage 4 cells and four passage 16 cells were karyotyped. C banding, RBG banding, and silver staining of the nucleolus organizing region (Ag-NOR) were performed as described earlier [22,23].

Estrogen and Progesterone Receptor Determination

Fresh tumor or cultured cells were frozen in liquid nitrogen and were transported to the Ligand Laboratory where the ER and PgR content was determined using the dextran–charcoal method [24,25]. The cultured cells were grown for receptor assays as described previously [15,26]. Tamoxifen citrate was generously provided by Leiras-Medica, Huhtamäki Oy, Turku, Finland. 17β-Estradiol was purchased from Sigma Chemical Company, St. Louis, Missouri.

Effects of Tamoxifen, Estradiol, and Epidermal Growth Factor on Cell Growth

In each experiment testing the effects of TAM on UM-EC-2 cells, the MCF-7 breast cancer cell line [27] was used as a TAM-sensitive control. The effects of TAM on MCF-7 were consistent with those reported in earlier publications [15,26] and are not illustrated in this article. Cells in logarithmically growing cultures were harvested with trypsin–EDTA, washed, resuspended, counted, and distributed to the wells of 96-well plates (Costar Corp., Cambridge, MA). Cultures were fed daily with cDMEM containing 5% FBS stripped free of steroids by treatment with dextran-coated charcoal (DCC) (D5 medium) [24]. After 3 days in culture the logarithmically growing cells were fed daily with D5 medium containing the appropriate concentrations of TAM or estradiol (E_2) prepared according to standard methods [15,26,28,29]. Control cultures were fed with D5 medium. To test the capacity of E_2 to prevent growth inhibition by TAM, cultures were fed simultaneously with E_2 (100 n*M*) and TAM (3.5 μM). For this experiment the control cultures were fed with D5 medium containing either E_2 (100 n*M*) alone or TAM (3.5 μM) alone. To determine whether UM-EC-2 cells recover from the growth inhibitory effect of TAM more rapidly in the presence of E2, cultures were treated with 5 μM TAM for 3 days; then the medium was removed and cultures were fed daily either with D5 medium containing 100 n*M* E_2 or D5 medium alone and cell counts were performed every other day. Epidermal growth factor was purchased from Collaborative Research, Inc., Cambridge, Massachusetts. To assess the effect of EGF on cell growth, EGF at a concentration of 1 n*M* was added to D5 medium and cultures were fed and harvested as described above.

RESULTS

Histology and in Vitro Culture

The histology of the tumor from the patient was read as a poorly differentiated adenocarcinoma with extensive areas of necrosis. The neoplasm contained sheets of loosely organized malignant cells with focal areas of attempted gland formation. The histology of tumors formed in nude mice by UM-EC-2 cells were also read as poorly differentiated adenocarcinoma with focal areas of attempted gland formation. Both the original tumor and those formed in nude mice by cultured UM-EC-2 cells had a similar lacy stromal pattern and variable cytoplasmic vacuolization that resulted in some areas with a clear cell appearance. In primary culture, UM-EC-2 cells grew slowly at first and were sufficiently confluent for passage only after 5 months in culture. The cells are small and grow in cell islands with a tendency to pile up in three-dimensional colonies. Examples of the histology of the patient's tumor, a tumor formed in an athymic mouse after inoculation of cultured UM-EC-2 cells, and the appearance of UM-EC-2 are shown in Fig. 1.

Cell Surface Antigen Expression

The cell surface antigen expression of UM-EC-2 cells is shown in Fig. 2. Rabbit antiserum to human β_2-microglobulin was used to detect class I histocompatibility antigen expression. This reagent binds strongly to UM-EC-2 cells (Fig. 2A). We previously observed in immunoperoxidase assays on tissue sections that normal endometrial glands express blood group antigens consistent with the donor's blood type (data not shown). The donor of the UM-EC-2 cell line is blood group O. Accordingly, there was no binding of antibodies to blood group A or B (not shown). However, UM-EC-2 cells do strongly express the H type 2 blood group antigen corresponding to blood type O as determined by binding of the H type 2-specific monoclonal antibody, UM-G10 [19] (Fig. 2B). UM-EC-2 cells do not express the A9 antigen defined by monoclonal antibody UM-A9 (Fig. 2C). This antigen is expressed by all squamous cell carcinomas and is found in the basal lamina of many glandular structures including endometrial glands but is expressed only by a small proportion of adenocarcinomas [18]. EGF receptor was strongly expressed on UM-EC-2 cells as determined by binding of the EGFR1 monoclonal antibody [21] (Fig. 2D). We found no reactivity of UM-EC-2 cells with antibodies to squamous cell-specific antigens [16,17] defined by autoantibodies from patients with pemphigus vulgaris or bulbous pemphigoid (not shown). This phenotype is consistent with that expressed by the UM-EC-1 endometrial carcinoma cell line [22].

Karyotype

The UM-EC-2 cultures contained two cell populations. One population was hypertetraploid and the other was hyperdiploid. The proportion of the two populations differed in lineages derived from separate primary flasks. Thus the population in the flask that was analyzed in passage 1 was predominantly hypertetraploid, with only two hyperdiploid cells. The cells in the flasks labeled passages 2, 4, and 16 were derived from a different primary culture flask and these were predominantly hyperdiploid (9/15 cells in culture P2, 7/7 in culture P4, and 3/4 in P16). Representative karyotypes are presented in Figs. 3 and 4. Rearrangements involving chromosomes 1, 2, 4, 5, 6, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 21, 22, and X were identified. Most of the rearrangements were common to both populations (Table 1 and Figs. 3 and 4), but some additional changes occurred in each population after the two populations became separate (Table

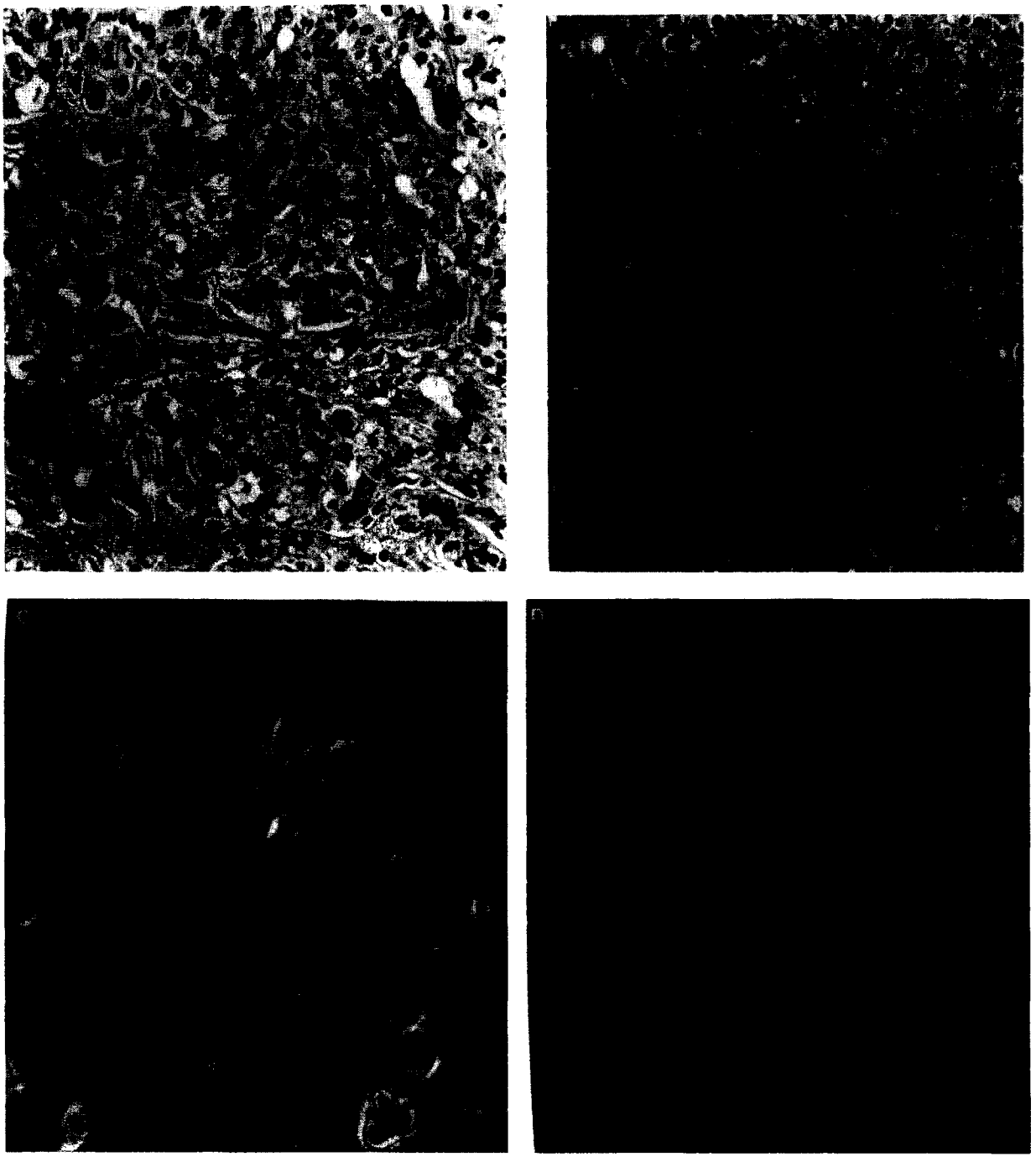

FIG. 1. Histology of the patient's tumor, a tumor formed in a nude mouse inoculated with UM-EC-2 cells, and appearance of cultured cells at passage 4. (A) High-power photomicrograph of a section of the primary poorly differentiated endometrial carcinoma, showing pleomorphism for cell size and shape, nested clusters of tumor cells, and areas of apparent lumen formation (arrows). (B) High-power photomicrograph of a section of a nude mouse tumor produced by passage 7 UM-EC-2 cells showing pleomorphism similar to that in the original tumor, nested clusters, and apparent lumen formation (arrows). (C) Inverted phase-contrast photomicrograph of passage 4 UM-EC-2 cells in culture Day 3 after passage (240×). (D) Same culture as in C, shown on Day 8 after passage (80×). The cells grow in islands and have a tendency to pile up in three-dimensional colonies, mimicking the nested appearance observed in tissue sections.

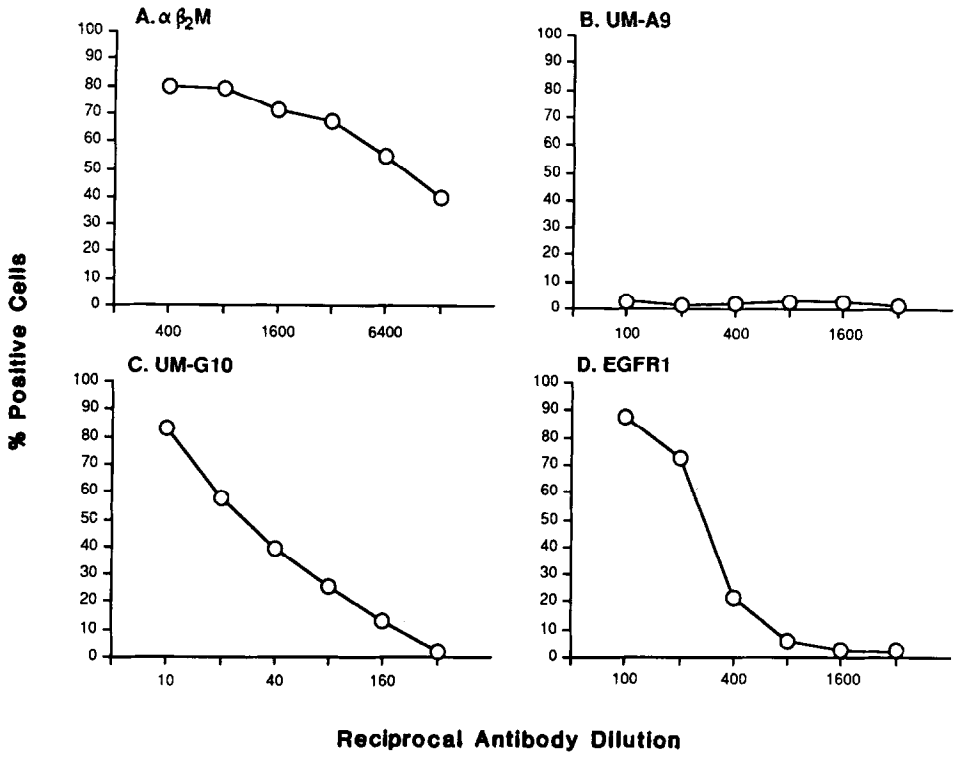

FIG. 2. Cell surface antigen phenotype of UM-EC-2 cells. Antigen expression was determined using hemadsorption assays on cells growing in plastic 96-well plates. (A) Rabbit antiserum to human β_2-microglobulin ($\alpha\beta_2$M). (B) Monoclonal antibody UM-A9 to an epithelial basement membrane antigen. (C) Monoclonal antibody UM-G10 to the H type 2 blood group precursor antigen [19]. (D) Commercial monoclonal antibody EGFR1 to the external domain of the epidermal growth factor receptor [21].

2). A postulated sequence of events in the evolution of the karyotype was derived by analyzing the number of copies of each chromosome in the hyperdiploid and hypertetraploid populations as described previously [23]. In some cases the differences in C banding between the homologs could be used to distinguish which chromosome was involved in the rearrangements. This postulated sequence is presented in Table 2. The rearrangements common to both populations are most readily appreciated from Figs. 3 and 4. These include del(2)(q), del(4)(q) (entire homolog lost in some cells of the hyperdiploid population), der(6)t(6;12), del(7)(p), del(8)(p), der(9)t(9;9), del(9)(q), i(dic(11)), der(12)t(12;21), del(13)(q), der(16)t(16;?), i(17p), inv(i(17q)), der(18)t(18;?), der(21)t(21;?), der(22)t(22;?), and der(X)t(X;15)(q22q15). In addition, there were from one to four inconsistent markers.

Receptor Content and Effects of Tamoxifen and Estradiol on Cell Growth

No ER or PgR were found either in the patient's tumor or in UM-EC-2 cells grown in culture. In spite of the absence of receptors, the cells were very sensitive to TAM. The growth inhibitory effect of 1–10 μM TAM on UM-EC-2 cells in logarithmic growth is illustrated in Fig. 5. After 4 days of daily feeding with 1 μM TAM, the growth of UM-EC-2 cells was retarded by 50% relative to the control cultures fed with medium alone. There was cytostasis (i.e., no increase in cell number) in cultures treated with 2.5 μM TAM, and in cultures fed concentrations of 5–10 μM there was significant cytotoxicity.

Estradiol (100 nM E_2), when given alone, moderately stimulated the growth of UM-EC-2 cells (Fig. 6). However, this concentration of E_2 did not block the growth inhibition caused by 3.5 μM TAM when the two agents were added to the cultures simultaneously (Fig. 6). Similarly, when cultures were fed 5 μM TAM to induce growth inhibition and then tested for recovery of logarithmic growth there was no difference between cells recovering in D5 medium or in D5 medium supplemented with 100 nM E_2 (Fig. 7).

Effect of Epidermal Growth Factor on Cell Growth

Moderately elevated expression of the EGF receptor was detected by binding of the EGFR1 antibody (Fig. 2). Korc *et al.* have reported that EGF inhibits growth of the RL95-2 endometrial carcinoma cell line [30].

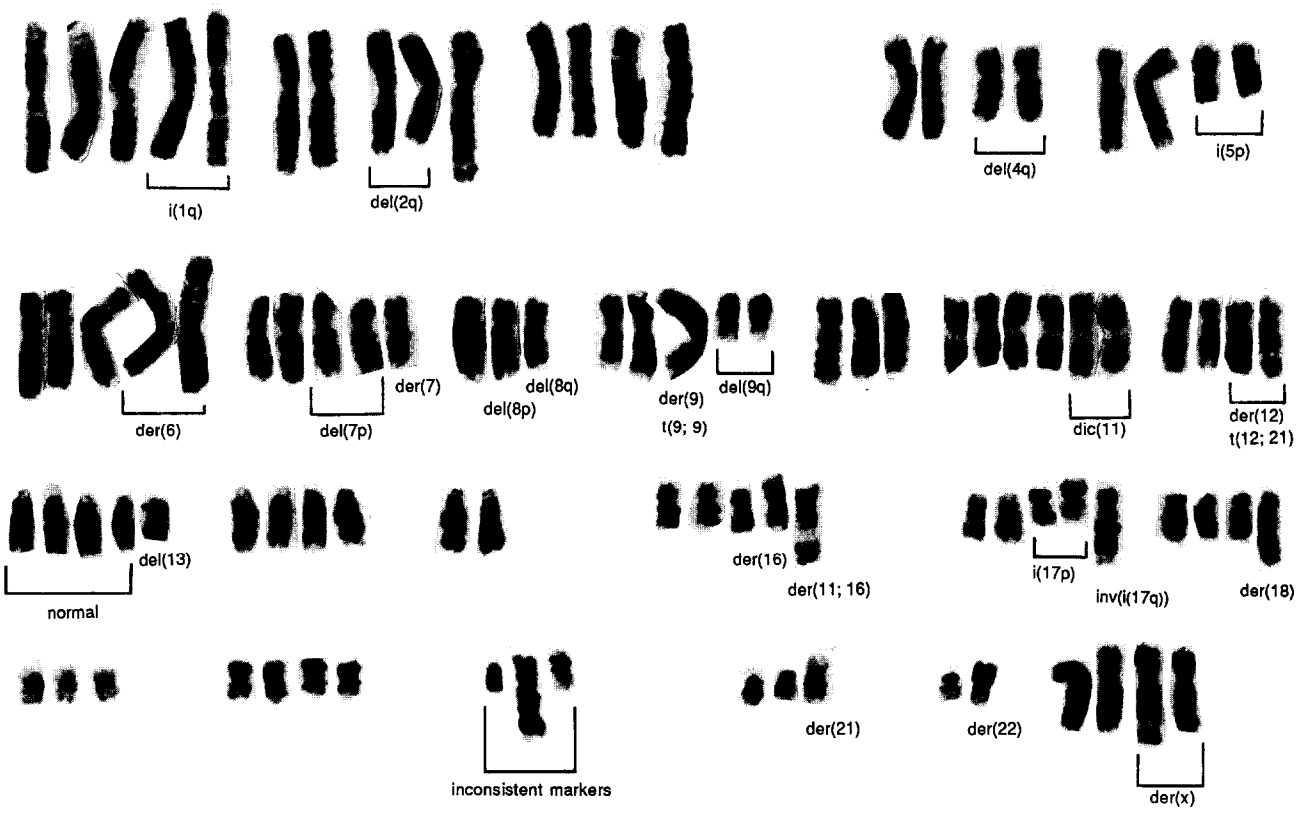

FIG. 3. Karyotype of a metaphase from UM-EC-2, passage 1. There are three normal copies of chromosome 1 and two i(1q). There are two normal chromosomes 2 and two del(2)(q), and in this cell there was an additional chromosome 2 with unidentified material attached to distal 2q. This was not seen in any other metaphase. There are four normal chromosomes 3, two normal chromosomes 4, and two del(4)(q). There are two normal chromosomes 5 and two i(5p). There are three normal chromosomes 6 and two der(6). There are two normal chromosomes 7, two del(7)(p), and one der(7). There is one normal chromosome 8, one del(8)(p), and one del(8)(q). There are two normal chromosomes 9, a der(9), and two del(9)(q). There are three normal chromosomes 10, four normal chromosomes 11, and two dic(11). There are two normal chromosomes 12 and two der(12). There are four normal chromosomes 13 and one del(13)(q). The four chromosomes 14 and the two chromosomes 15 are normal. There are three normal chromosomes 16, one der(16)t(16;?)(p13.3;?), and one der(16)t(11;16)(q13;q24). There are two normal chromosomes 17, two i(17p), and one inv(i(17q)). There are three normal chromosomes 18 and one der(18)t(18;?)(q22;?). There are three normal chromosomes 19, four normal chromosomes 20, two normal chromosomes 21, and one der(21). There is one normal chromosome 22 and one der(22). There are two normal late-replicating (genetically inactivated) X chromosomes and two early replicating (genetically active) der(X). There were three marker chromosomes in this cell. The marker in the center is possibly a distorted inv(i(17q)); most hypertetraploid cells had two copies of this chromosome.

Therefore, it was of interest to examine the effect of EGF on the growth of UM-EC-2 cells. EGF (at a concentration based on the experiments reported by Korc *et al.* [30]) was added to logarithmically growing cultures starting on Day 4 and cell growth was monitored for the next 11 days. As shown in Fig. 6, EGF had little effect on the growth of the cells at 3 days (Day 7), but thereafter a progressively greater difference in cell number between EGF-treated and control cultures was noted. By Day 15 the growth of EGF-treated cultures was reduced to 73% of that in the control cultures.

DISCUSSION

UM-EC-2 is the second of three cell lines derived in our laboratory from patients with poorly differentiated endometrial adenocarcinoma. UM-EC-3 has not yet been fully characterized but some interesting comparisons can be made between the other two lines. The tumors from which UM-EC-1 and UM-EC-2 cell lines were established showed aggressive clinical behavior leading to metastatic spread in the donors. Characterization of these cell lines revealed both similarities and differences. Both lines produce tumors in nude mice that resemble the donors' neoplasms. Furthermore, both lines strongly express class 1 histocompatibility antigens and the EGF receptor (expression of EGFR by UM-EC-1 is unpublished, Carey *et al.*). The expression of the EGF receptor was fairly high on UM-EC-2 when compared to normal fibroblasts and keratinocytes. The latter cell types show no reactivity with the EGFR1 antibody at dilutions of 1/100 or greater. However, the expression of this antigen

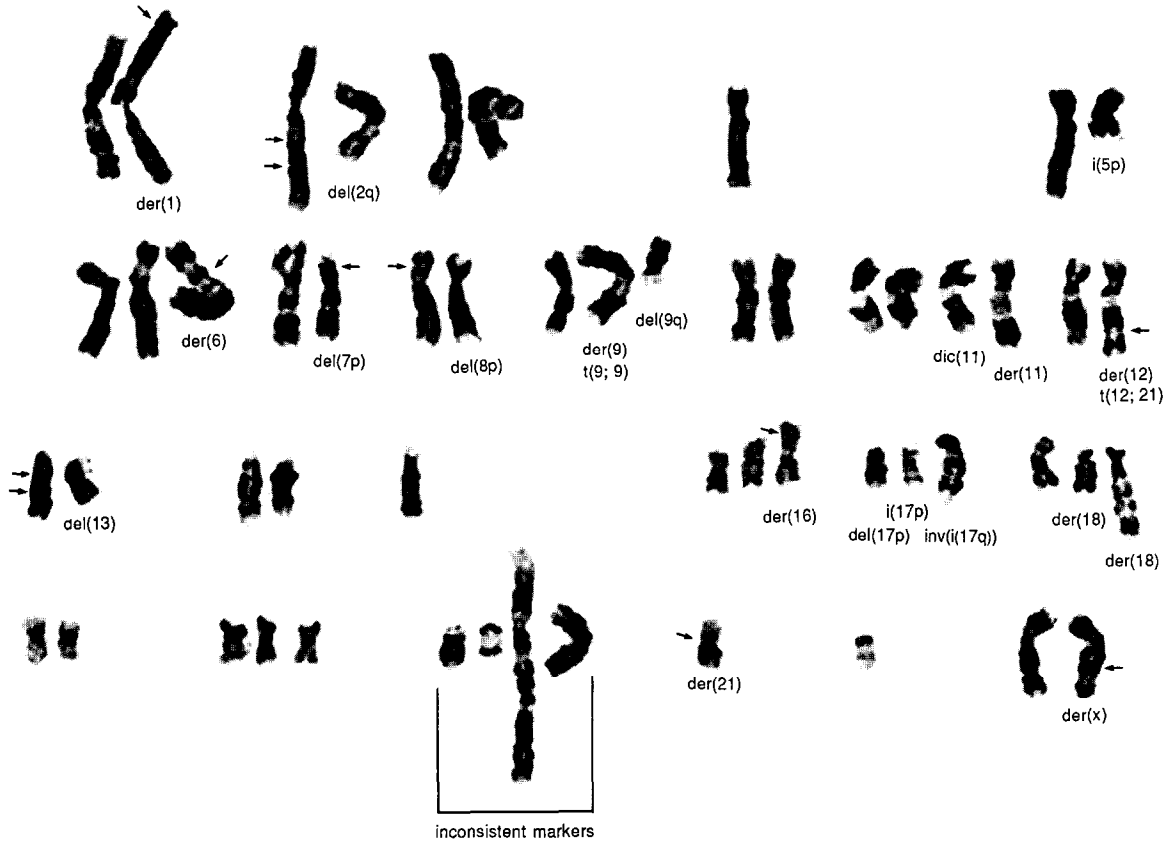

FIG. 4. Karyotype of a metaphase from UM-EC-2, passage 2. There is one normal chromosome 1 and one der(1). The arrow points to the breakpoint on the der(1). There is one normal chromosome 2 and one del(2)(q). The arrows point to the region on the normal two that is lost in the del(2)(q). Both chromosomes 3 appear normal. There is one normal chromosome 4. The del(4)(q) is not represented in this karyotype. There is one normal chromosome 5 and one i(5p). There are two normal chromosomes 6 and one der(6). The arrow shows the point on 6 where the translocated material from 12q is attached. There is one normal chromosome 7 and one del(7)(p) with an arrow showing the region of the deleted material. There is one normal chromosome 8 (with an arrow pointing to the band that is lost from the deleted 8) and one del(8)(p). There is one normal chromosome 9, one der(9), and one del(9)(q). There are two normal chromosomes 11, one dic(11), and one der(11). There is one normal chromosome 12 and one der(12), with an arrow pointing to the breakpoint between 12 and the translocated 21 material. There is one normal chromosome 13 (with arrows marking the region that is lost from the other chromosome) and one del(13). There are two normal chromosomes 14 and a single normal 15. There are two normal chromosomes 16 and one der(16)t(16;?)(p13;?) (arrow indicates the breakpoint for the translocated material). There is no normal chromosome 17 in this cell (most cells had one normal 17). There is a del(17)(p11), an i(17p), and an inv(i(17q)). There is a normal chromosome 18, a der(18)t(18;?)(p11.1;?), and a der(18)t(18;?)(q22;?). There are two normal chromosomes 19, three normal chromosomes 20, and one der(21), with an arrow marking the junction with the unidentified material. The normal chromosome 21 is missing from this and some other cells. There is one normal chromosome 22. There is one normal X chromosome and one der(X) and from a t(X;15), with an arrow marking the junction between the two chromosome segments. There are four unidentified chromosomes in this metaphase (inconsistent markers).

is relatively low on UM-EC-2 when compared to the EGFR-amplified breast cancer cell line MDA-MB-468, which has approximately 10^6 receptors and a 50% end-point titer of 1/12,800 with the EGFR1 antibody (T. Carey *et al.*, unpublished data). The two lines differ with respect to blood group antigen expression. UM-EC-1, which was derived from a blood group A individual, expresses neither the A nor the H precursor antigen, whereas UM-EC-2, which was derived from a blood group O individual, expresses the H antigen strongly. As expected, neither endometrial carcinoma cell line showed reactivity with antibodies to squamous cell antigens.

Cytogenetic analyses performed previously with UM-EC-1 [22] and in this study with UM-EC-2 suggest that endometrial carcinomas may have nonrandom chromosome abnormalities. UM-EC-2 has a much more complicated karyotype than UM-EC-1. However, numerous similarities were present in each line. Both lines had loss of nearly all of chromosome 4, a derived chromosome 8 with breakpoints in the short arm at band p23 in UM-EC-1 and band p22 in UM-EC-2, a deletion of the long

TABLE 1
Comparison of the Karyotypes of UM-EC-2, Passages 1, 2, and 4[a]

Chromosome	UM-EC-2-P1 CN[b] = 109 (hyperteraploid population) Consensus karyotype	UM-EC-2-P2 CN = 55 (hyperdiploid) Number of chromosome copies per cell relative to the consensus karyotype	UM-EC-2-P4 CN = 53 (hyperdiploid) Number of chromosome copies per cell relative to the consensus karyotype
1	4 (some 3),+ two i(1)(p11)	1,+der(1)t(1;?)(p36.3;?)	Same
2	4 (some 3), + two del(2)(q21q31)	2 (some 1),+del(2)(q21q31)	Same
3	4 (some cells 3)	2	Same
4	4 (some 3), + two del(4)(q23q33)(some 3)	1,+del(4)(q23q33)(some 0)	1
5	2, + two i(5p)	1,+i(5p)(some 2, some 0)	1,+i(5p)
6	4 (some 3),+ two dic(6) t(6;12)(p24;q14)(some 1)	2, +dic(6)t(6;12)(p24;q14)	Same
7	2, + two del(7)(p22p15), + der(7)t(7;?)(q11.2;?)	1, +del(7)(p22p15)	1, +del(7)(p22p15) + del(7)(q22q36)(some 0)
8	2, + two del(8)(p22p12)(some cells 1) + del(8)(q24.1q24.3)	1, +del(8)(p22p12)	Same
9	2, + two del(9)(q13) + der(9)t(9;9)(p21;q13)	1, +del(9)(q13), + der(9)t(9;9)(p21;q13)	Same
10	3 (some cells 4)	2	
11	4, + two dic (11)(q13) + del(11)(p13) (some cells 0)	2, + dic (11)(q13) + dic der(11;12)(q14;q14)	Same
12	2, + two der(12)t(12;21)(q22;q11.2)	1, +der(12)t(12;21)(q22;q11.2)	Same
13	4 (some cells 3) + two del(13)(q14q21)	2, del(13)(q14q21)	Same
14	4 (some cells 1)	2	Same
15	2	1	Same
16	3 (some cells 2, some 1) + two der(16)t(16;?)(p13.3;?) + two der(16)t(11;16)(q13;q24)	2,+ der(16)t(16;?)(p13.3;?)	Same
17	2, + two inv(i(17q))t(q21;q25) + two i(17p)	1, +inv(i(17q))t(q21;q25) + i(17p)	Same
18	4 (some cells 3, some 2) + two der(18)t(18;)(?q22;?) (some 1)	1, +der(18)t(18;?)(?q22;?) + der(18)t(18;?)(p11.1;?)	Same
19	4 (some cells 3, some 2)	2	Same
20	5 (some cells 4, some 3)	3 (some cells 4, some 2)	Same
21	2 or 3 (some cells 1) + two der(21)t(21;?)(p11;?) (some cells 1 some 0)	1 (some cells 0) + der(21)t(21;?)(p11;?) (some cells 0)	Same
22	2, + der(22)t(22;?)(p11;?) (some cells 0)	1, +der(22)t(22;?)(p11;?) (some cells 0)	Same
Unidentified	2–7	1–2	Same
X	2, + two der(X)t(X;15)(q22;q15)	1, + der(X)t(X;15)(q22;q15)	Same

[a] Passages 1 and 2 were separate cultures; passage 4 came from passage 2.
[b] Consensus number.

arm of chromosome 9 with breakpoints at q11 (UM-EC-1) and q13 (UM-EC-2), a common breakpoint at 12q22, loss of chromosome 13 (the entire homolog in UM-EC-1 and q14-q21 in UM-EC-2), and deletion or rearrangement of 18q with breakpoints in adjacent bands at q11.2 and q21.3 in UM-EC-1 and at q22 in UM-EC-2; both lines also had a translocation involving chromosome 22p11. In fact, except for a difference in the breakpoints on chromosome 1 and the changes in chromosome 19, all of the chromosome abnormalities in UM-EC-1 were also present in UM-EC-2. The common affected chromosome bands in these cell lines provide the starting points for the molecular genetic analysis of the genes that are routinely altered in poorly differentiated endometrial carcinoma. Furthermore, as other less advanced cancers are evaluated it will be possible to distinguish which of these changes are related to the highly malignant behavior exhibited by poorly differentiated tumors.

In ER-positive cells 100 n*M* E_2 is generally at the upper limit of the stimulatory range and higher concentrations usually inhibit cell growth. For example, growth stimulation of the ER-positive MCF-7 cell line occurs at E_2

TABLE 2

Postulated Sequence of Cytogenetic Events in the Development of the Endometrial Carcinoma from Which the UM-EC-2 Lines Were Established

Normal karyotype	Early changes in population (P2)	Hyperdiploid changes	Further changes	Tetraploidy	Further changes	Final hypertetraploid population
1 (small C band)		1		1,1		1,1
1 (large C band)		1,1	→ 1,i(1q)	1,1, i(1q),i(1q)		1,1,two i(1q)
	+1	der(1)t(1;?)(p36.3;?)→	−der(1)t(1;?)			
2	+2	2,2		2,2,2,2		2,2,2,2
2 →	del(2)(q21q31)	del(2)(q21q31)		del(2),del(2)		two del(2)
3		3		3,3		3,3
3		3		3,3		3,3
4		4		4		4,4
4 →	del(4)(q23q33)	del(4)(q23q33) (some cells −del(4))		del(4),del(4)		two del(4)
5		5		5,5		5,5
5 →	i(5p),i(5q)	i(5p)		i(5p.i(5p)		two i(5p)
6	+6	6,6		6,6,6,6		6,6,6,6
6 →	dic(6)t(6;12)(p24;q14)	dic(6)t(6;12)		two dic(6)t(6;12)		two dic(6)t(6;12)
7		7		7,7	⇄ +7	7,7
					↘ der(7)t(7;?)(q11.2;?)	der(7)t(7;?)
7	del(7)(p22p15)	del(7)(p22p15)		two del(7)(p22p15)		two del(7)(p22p15)
8		8		8,8	⇄ del(8)(q24.1q24.3)	8,8,del(8q)
					↘ +8	
8 →	del(8)(p22p12)	del(8)(p22p12)		del(8p),del(8p)		two del(8p)
9 break at 9q13→ (small C band)	del(9)(q13)	del(9)(q13)		del(9),del(9)		two del(9)
9 ⇄ (large C band)	+9	9		9,9		9,9
↘	der(9)t(9;9)(p21;q13) with loss of p21-pter	der(9)t(9;9)		der(9),der(9)	→ −der(9)	der(9)
10		10		10,10		10,10
10		10		10,10		10,10
11	+11	11,11 11		11,11,11,11	⇄ 11	11,11,11,11,
					↘ del(11)(q13)	del(11q13)
11 ⇄ 11	dic der(11)t(11;12)(q14q14)	dic der(11)t(11;12)	→ −dic der(11)t(11;12)			
↘ +11→	dic(11)(q13)	dic(11)(q13)		dic(11)(q13),dic(11)(q13)		two dic(11)(q13)
(the 11q13-qter segment went to chromosome 16q)						
12 →	der(12)t(12;21)	der(12)t(12;21)		der(12);der(12)		der(12);der(12)
12 ⇄	12	12			12,12	
↘ 12,12						
	+12	der(11)t(11;12) (see chromosome 11)	→ −der(11)t(11;12)			
13 ⇄	13	13,13		13,13,13,13		13,13,13,13
↘	+13					
13 →	des(13)(q14q21)	del(13)		des(13);del(13)		two del(13)
14		14		14,14		14,14
14		14		14,14		14,14
15		15		15,15		15,15
15 →	der(X)t(X15)(q22q15)					
16 ⇄ 16		16		16,16	+16	16,16,16
↘ +16→	der(16)t(11;16)(q13q24)	der(16)t(16;?) one of 15 cells		two der(16)t(16;?)		two der(16)t(16;?)
16 →	der(16)t(16;?)(p13.3;?)					
17 ⇄ i(17p)		17		17,17		17,17
↘ (i(17q))		i(17p)		i(17p),i(17p)		i(17p),i(17p)
	inv(i(17q))(q21q24)	inv(i(17q))		inv(i(17q)),inv(i(17q))		two inv(i(17q))
18 ⇄	18	18		18,18		18,18
↘	+18→der(18) t(18;?)(p11.1;?)	der(18)t(18;?) (some cells 0)	→ −der(18)t(18;?)			
18	der(18;?)t(q22;?)	der(18)t(18;?)(q22;?)		two der(18)t(18;?)(q22;?)		two der(18)
19		19		19,19		19,19
19		19		19,19		19,19
20 ⇄	20	20,20		20,20		20,20
↘	+20					
20		20		20,20		20,20
21 ⇄	21	21		21,21		21,21
↘	+21→der(12;21) (see chromosome 12)					
21 →	der(21)t(21;?)(p11;?)	der(21)		two der(21)		two(der(21)
22		22		22,22		22,22
22 →	der(22)t(22;?)(p11;?)	der(22)		der(22),der(22)→	−der(22)	der(22)
X (late replicating)		X		X		X,X
X (early replicating)→	der(X)t(X;15)(q22q15)	der(X)		der(X),der(X)		der(X),der(X)

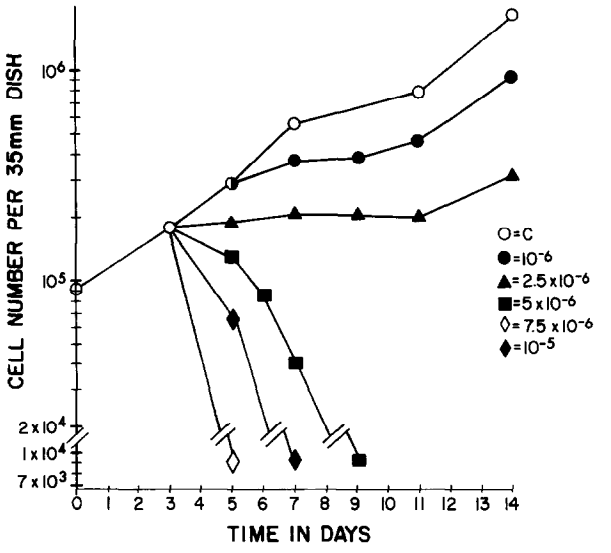

FIG. 5. Cell growth curves showing the effects of TAM on UM-ED-2 cells. Experiments were performed in medium containing 5% DCC-treated FBS. Daily feeding with medium containing TAM was begun on Day 3. Control (open circles), 1 μM TAM (solid circles), 2.5 μM TAM (solid triangles), 5 μM TAM (solid squares), 7.5 μM TAM (solid diamonds), and 10 μM TAM (open diamonds).

concentrations ranging from 1 to 100 nM, whereas growth inhibition is observed at 1 μM E_2 [28]. Steroid hormone receptors could not be detected in UM-EC-2 cells. However, 100 nM E_2 stimulated the growth of these cells by an as yet unknown mechanism. At this time we cannot rule out that in these cells there may be an induction of ER in the presence of high concentrations of E_2 that then mediates the growth stimulatory effects we observed.

Poorly differentiated and recurrent endometrial carcinomas often lack steroid receptors and exhibit a poor response to progestins [5–7,31]. We recently demonstrated that TAM caused growth inhibition in four ER- and PgR-negative, MPA-resistant cell lines derived from moderately or poorly differentiated endometrial carcinomas [15]. One of those cell lines, UM-EC-1 [22], was established in our laboratory from a patient who had progression of her tumor during postoperative MPA therapy, but experienced an objective clinical response to subsequent TAM treatment [15]. Thus, there was a good correlation between our *in vitro* study and the clinical response in that patient.

TAM also caused a dose-dependent growth inhibition of logarithmically growing UM-EC-2 cells *in vitro*. In fact UM-EC-2 is as sensitive to TAM as the MCF-7 breast cancer cell line. MCF-7 is used in many laboratories to study antihormone effects of TAM [32–34] and routinely exhibits cessation of logarithmic growth in 5

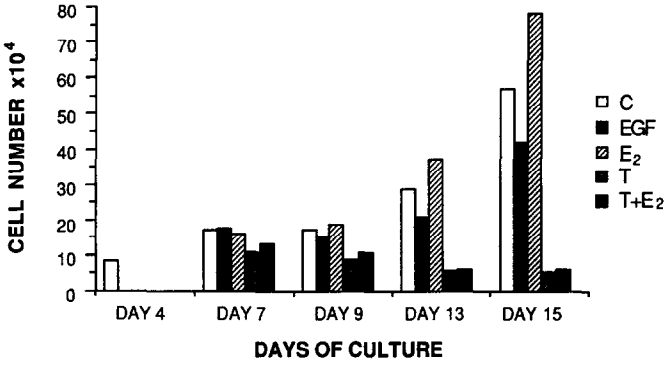

FIG. 6. Effects of hormonal agents on the growth of UM-EC-2 cells. Cells were plated and allowed to resume logarithmic growth. Starting on Day 4 the cells were fed daily with either D5 medium (control = C) or with D5 medium containing 1 nM EGF (EGF), 100 nM E_2 (E_2), 3.5 μM TAM (T), or 3.5 μM TAM and 100 nM E_2 (T + E_2). Cell counts of three replicate cultures were performed on the days indicated. Results are expressed as cell number per 35-mm dish.

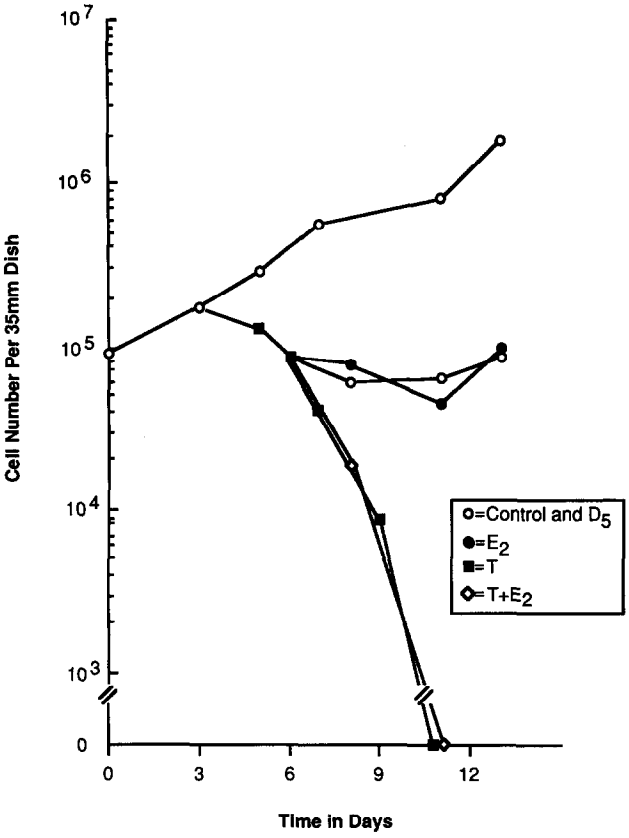

FIG. 7. Reversibility of the growth inhibitory effects of tamoxifen on UM-EC-2 cells. Cells in logarithmically growing cultures were harvested and plated at 8 × 10^5 cells per well and fed for 3 days with D5 medium. Some wells were then fed with medium containing 5 μM TAM for 3 days to inhibit growth (solid squares). On Day 7 groups of wells were fed with D5 medium alone, with D5 medium containing 100 nM E_2, with D5 medium containing 5 μM TAM (T), or with D5 medium containing both 5 μM TAM and 100 nM E_2 (T + E_2). Cell counts were performed on three replicate cultures on the days indicated.

μM TAM [15,26,28,29]. Similar levels of growth inhibition were obtained in UM-EC-2 cultures with 2.5–3.5 μM TAM. In UM-EC-2 cultures the growth inhibitory effect of TAM was not reversed by E_2; likewise, cells removed from TAM did not recover more rapidly from growth inhibition in the presence of E_2. The inability of E_2 to reverse the effects of TAM is consistent with absence of measurable levels of the ER. These findings are also in agreement with our previous data obtained with other ER-negative cell lines including UM-EC-1 [15,26,28,29] and support the concept of an ER-independent mechanism mediating part of the growth inhibitory effects of TAM. Clinical studies show an overall response rate of 20% to TAM in patients with advanced or recurrent endometrial carcinoma [9–14]. Steroid hormone receptor content of the tumors is unknown in most of the reports, and so it is not possible to draw any conclusions of the correlation of the response to TAM and the ER or PgR expression of the tumors. There is extensive literature showing evidence for distinct binding sites for nonsteroidal antiestrogens [35–37]; however, the role of these antiestrogen binding sites in mediating the growth inhibition caused by TAM has not been demonstrated [36,37]. We have not yet determined whether there are specific antiestrogen binding sites in our ER-negative, TAM-sensitive cell lines. The mechanism by which TAM binds and regulates growth in ER-negative cells remains to be determined. The donor of UM-EC-2 died before we obtained the *in vitro* data showing how sensitive this tumor is to TAM. This is unfortunate because the *in vitro* results and the beneficial effects of TAM in the donor of UM-EC-1 suggest that TAM may have altered the course of her disease.

EGF has growth stimulatory effects on normal cells, but in tumors where the receptor is overexpressed, EGF can be growth inhibitory. In fact, EGF has been reported to inhibit the proliferation of RL95-2 human endometrial carcinoma cells [30,38], and under similar conditions UM-EC-2 cells were also susceptible to growth inhibition by EGF *in vitro*.

It has recently been shown that hormonal agents can modulate the levels of polypeptide growth factors and their receptors [30–43] and it has been suggested that growth factors may participate in postreceptor effects of estrogens and antiestrogens. Receptors for EGF are present in both normal [44,45] and malignant [30] human endometrial cells. It will be of interest to determine whether there is a link between the effects of steroid and polypeptide hormones on cell growth. UM-EC-2 cells provide a good model in which to study the interactions between growth factors, growth factor receptors, and growth regulatory antihormones such as TAM to better understand the mechanisms by which tumor growth may be controlled.

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REFERENCES

1. Kohorn, E. I. Gestagens and endometrial carcinoma. *Gynecol. Oncol.* **4,** 398–411 (1976).
2. Kauppila, A. Progestin therapy of endometrial, breast, and ovarian carcinoma: A review of clinical observations, *Acta Gynecol. Scand.* **63,** 441–450 (1984).
3. Aalders, J. G., Abeler, V., and Kolstad, P. Clinical (stage III) as compared to subclinical intrapelvic extrauterine tumor spread in endometrial carcinoma: A clinical and histopathological study of 175 patients, *Gynecol. Oncol.* **17,** 64–74 (1984).
4. Annual Report of the Treatment in Gynaecological Cancer, International Federation of Gynecology and Obstetrics, Stockholm, Vol. 20 (1988).
5. Ehrlich, C. E., Young, P. C. M., and Cleary, R. E. Cytoplasmic progesterone and estradiol receptors in normal, hyperplastic and carcinomatous endometria: Therapeutic implications, *Amer. J. Obstet. Gynecol.* **141,** 539 (1981).
6. Creasman, W. T., McCarty, K. S., Barton, T. K., and McCarty, K. S., Jr. Clinical correlation of estrogen- and progesterone-binding proteins in human endometrial adenocarcinoma, *Obstet. Gynecol.* **55,** 363–370 (1982).
7. Kauppila, A. J. I., Isotalo, H. E., Kivinen, S. T., and Vihko, R. K. Prediction of clinical outcome with estrogen and progestin receptor concentrations and their relationship to clinical and histopathological variables in endometrial cancer, *Cancer Res.* **46,** 5380–5384 (1986).
8. Swenerton, K. D., Shaw, D., White, G. W., and Boyes, D. A. Treatment of advanced endometrial carcinoma with tamoxifen, *N. Engl. J. Med.* **301,** 105 (1979).
9. Bonte, J., Ide, P., Billiet, G., and Wynants, P. Tamoxifen as a possible chemotherapeutic agent in endometrial carcinoma, *Gynecol. Oncol.* **11,** 140–141 (1981).
10. Hald, I., Salimtschik, M., and Mouridsen, H. T. Tamoxifen treatment of advanced endometrial carcinoma: A phase II study, *Eur. J. Gynecol. Oncol.* **4,** 83–87 (1983).
11. Slavik, M., Petty, W. M., Blessig, J. A., Creasman, W. T., and Homesley, H. D. Phase II clinical study of tamoxifen in advanced endometrial adenocarcinoma: A Gynecologic Oncology Group study, *Cancer Treat. Rep.* **68,** 809–811 (1984).
12. Swenerton, K. D., Chrumka, K., Paterson, A. H. G., and Jackson, G. C. Efficacy of tamoxifen in endometrial cancer, in *Progress in cancer research and therapy* (F. Bresciani, Ed.), Raven Press, New York, Vol. 31, pp. 417–424 (1984).
13. Edmonson, J. H., Krook, J. E., Hilton, J. F., Long, H. J., III, Cullinan, S. A., Everson, L. K., and Malkasian, G. D. Ineffectiveness of tamoxifen in advanced endometrial carcinoma after failure of progestin treatment, *Cancer Treat. Rep.* **70,** 1019–1020 (1986).
14. Quinn, M. A., and Campbell, J. J. Tamoxifen therapy in advanced/recurrent endometrial carcinoma, *Gynecol. Oncol.* **32,** 1–3 (1989).

15. Grenman, S. E., Roberts, J. A., England, B. E., Grönroos, M., and Carey, T. E., In vitro growth regulation of endometrial carcinoma cells by tamoxifen and medroxyprogesterone acetate, *Gynecol. Oncol.* **30,** 239–250 (1988).
16. Carey, T. E. Establishment of epidermoid carcinoma cell lines, in *Head and neck cancer* (R. E. Wittes, Ed.), London, pp. 287–314 Wiley (1985).
17. Carey, T. E., Kimmel, K. A., Schwartz, D. R., Richter, D. E., Baker, S. R., and Krause, C. J. Antibodies to human squamous cell carcinoma, *Otolaryngol.–Head Neck Surg.* **91,** 482–491 (1983).
18. Kimmel, K. A., and Carey, T. E. Altered expression in squamous cell carcinoma of an orientation restricted epithelial antigen detected by monoclonal antibody A9, *Cancer Res.* **46,** 3614–3623 (1986).
19. Kimmel, K. A., Carey, T. E., Judd, W. J., and McClatchey, K. D. Monoclonal antibody (G10) to a common antigen of human squamous cell carcinoma: Binding of the antibody to the H type 2 blood group determinant, *J. Natl. Cancer Inst.* **76,** 9–19 (1986).
20. Jones, C., Kimmel, K. A., Carey, T. E., Miller, Y. E., Lehman, D. W., and MacKenzie, D. Further studies on a hybrid cell-surface antigen associated with human chromosome 11 using a monoclonal antibody, *Somat. Cell Genet.* **9,** 489–496 (1983).
21. Waterfield, M. D., Mayes, E. L., Stroobant, P., Bennett, P. L. P., Young, S., Goodfellow, P. N., Banting, G. S., and Ozanne, B. A monoclonal antibody to the human epidermal growth factor receptor, *J. Cell Biochem.* **20,** 149–161 (1982).
22. Grenman, S. E., Van Dyke, D. L., Worsham, M. J., del Rosario, F., Roberts, J. A., McClatchey, K. D., Schwartz, D., Babu, R., and Carey, T. E. UM-EC-1, a new hypodiploid human cell line derived from a poorly differentiated endometrial cancer, *Cancer Res.* **48,** 1864–1873 (1988).
23. Grenman, S. E., Van Dyke, D. L., Worsham, M. J., England, B., Hopkins, M., McClatchey, K. D., Grenman, R., and Carey, T. E. Phenotype characterization, Karyotypic analysis and in vitro tamoxifen sensitivity of new ER-negative vulvar carcinoma cell lines-UM-SCV-1A and UM-SCV-1B. *Int. J. Cancer,* in press.
24. Bonne, C., and Raynaud, J.-P. Assay of androgen binding sites by exchange with methyltrienolone (R1881), *Steroids* **27,** 497–507 (1976).
25. Virolainen, E., Vanharanta, R., and Carey, T. E. Steroid hormone receptors in human squamous carcinoma cell lines, *Int. J. Cancer* **33,** 19–25 (1984).
26. Grenman, S. E., Shapira, A., and Carey, T. E. In vitro response of cervical cancer cell lines CaSki, HeLa and ME180 to the antiestrogen tamoxifen, *Gynecol. Oncol.* **30,** 228–238 (1988).
27. Soule, H. D., Vasquez, J., Long, A., and Alberts, B. M. A human cell line from a pleural effusion derived from a breast carcinoma, *J. Natl. Cancer Inst.* **51,** 1409–1416 (1973).
28. Shapira, A., Virolainen, E., Jameson, J. J., Ossakow, S. J., and Carey, T. E. Growth inhibition of laryngeal UM-SCC cell lines by tamoxifen, *Arch. Otolaryngol. Head Neck Surg.* **112,** 1151–1158 (1986).
29. Grenman, R., Virolainen, E., Shapira, A., and Carey, T. E. In vitro effects of tamoxifen on UM-SCC head and neck cancer cell lines: Correlation with the estrogen and progesterone receptor content, *Int. J. Cancer* **39,** 77–81 (1987).
30. Korc, M., Padilla, J., and Grosso, D. Epidermal growth factor inhibits the proliferation of a human endometrial carcinoma cell line, *J. Clin. Endocrinol. Metab.* **62,** 874–880 (1986).
31. Quinn, M. A., Cauchi, M., and Fortune, D. Endometrial carcinoma: Steroid receptors and response to medroxyprogesterone acetate. *Gynecol. Oncol.* **21,** 314–319 (1985).
32. Lippman, M., Polan, G., and Huff, K. The effects of estrogens and antiestrogens on hormone responsive human breast cancer in long-term tissue culture, *Cancer Res.* **35,** 4595–4601 (1976).
33. Sutherland, R. L., Hall, R. E., and Taylor, I. W. Cell proliferation kinetics of MCF-7 human mammary carcinoma cells in culture and effects of tamoxifen on exponentially growing and plateau-phase cells, *Cancer Res.* **43,** 3998–4006 (1983).
34. Katzenellenbogen, B. S., Norman, M. J., Eckert, R. L., Peltz, S. W., and Mangel, W. F. Bioactivities, estrogen–receptor interactions, and plasminogen activator-inducing activities of tamoxifen and hydroxytamoxifen isomers in MCF-7 human breast cancer cells, *Cancer Res.* **44,** 112–119 (1984).
35. Sutherland, R. L., Murphy, L. C., Foo, M. S., Green, M. D., and Whyburne, A. M. High-affinity anti-oestrogen binding site distinct from the oestrogen receptor. *Nature (London)* **288,** 273–275 (1980).
36. Miller, M. A., and Katzenellenbogen, B. Characterization and quantitation of antiestrogen binding sites in estrogen receptor-positive and -negative human breast cancer cell lines. *Cancer Res.* **43,** 3094–3100 (1983).
37. Sheen, Y. Y., Simpson, D. D. M., and Katzenellenbogen, B. S. An evaluation of the role of antiestrogen-binding sites in mediating growth modulatory effects of antiestrogens: Studies using t-butylphenoxyethyl diethylamine, a compound lacking affinity for the estrogen receptor, *Endocrinology* **117,** 561–564 (1985).
38. Korc, M., Haussler, C. A., and Trookman, N. S. Divergent effects of epidermal growth factor and transforming growth factors on a human endometrial carcinoma cell line, *Cancer Res.* **47,** 4909–4914 (1987).
39. Osborne, C. K., Hamilton, B., and Livington, R. B. Epidermal growth factor stimulation of human breast cancer cells in culture, *Cancer Res.* **40,** 2361–2366 (1980).
40. Mukku, V. R., and Stancel, G. M. Regulation of epidermal growth factor receptor by estrogen, *J. Biol. Chem.* **260,** 9820–9824 (1985).
41. Knabbe, C., Lippman, M. E., Wakefield, L. M., Flanders, K. C., Kasid, A., Derynck, R., and Dickson, R. B. Evidence that transforming growth factor β is a hormonally regulated negative growth factor in human breast cancer cells, *Cell* **48,** 417–428 (1987).
42. Dickson, R. B., McManaway, M. E., and Lippman, M. E. Estrogen-induced factors of breast cancer cells partially replace estrogen to promote tumor growth, *Science* **232,** 1540–1543 (1986).
43. Vignon, F., Bouton, M. M., and Rochefort, H. Antiestrogens inhibit the mitogenic effect of growth factors on breast cancer cells in the total absence of estrogens, *Biochem. Biophys. Res. Commun.* **146,** 1502–1508 (1987).
44. Hofmann, G. E., Rao, C. V., Barrows, G. H., Schultz, G. S., and Sanfilippo, J. S. Binding sites for epidermal growth factor in human uterine tissues and leiomyomas, *J. Clin. Endocrinol. Metab.* **58,** 880–884 (1984).
45. Chegini, N., Rao, Ch. V., Wakins, N., and Sanfilippo, J. S. Binding of ^{125}I-epidermal growth factor to human uterus. *Cell Tissue Res.* **246,** 543–548 (1986).