Growth Inhibition by Progestins in a Human Endometrial Cancer Cell Line with Estrogen-independent Progesterone Receptors

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ABSTRACT

The presence of estrogen-independent progesterone receptors (PgR) was demonstrated in a subline of a human endometrial cancer cell line, Ishikawa cells, although the original Ishikawa cells contained estrogen-inducible PgR. Scatchard plot analysis of cytoplasmic binding data in our subline (IK-90) revealed a high affinity binding site for R5020 (K0, 1.0 nM) with maximum binding sites of 158 fmol/mg protein. Competition experiments showed a binding specificity similar to that of typical PgR. By low-salt sucrose gradient centrifugation, radioactive 8S and 4S peaks were found. The addition of 1 µM progesterone in culture medium resulted in a rapid nuclear translocation of cytoplasmic PgR. In contrast to the original cells, estrogen receptors could not be detected in IK-90 cells, and an addition of 17β-estradiol (10 nM) to culture medium failed to increase PgR. Accumulation of glycogen in cytoplasm of IK-90 cells in response to R5020 (0.1-1 µM) was observed by periodic acid-Schiff staining. The addition of R5020 to culture medium (0.1-1 µM) also caused a marked decrease in the growth of IK-90 cells, whereas the other steroids including 17β-estradiol, tamoxifen, testosterone, and cortisol had no significant effects. These results demonstrate for the first time the presence of a progesterin-responsive human endometrial cancer cell line that contains estrogen-independent functional PgR. IK-90 cells appear to be an ideal model for studying the mechanism of the antiproliferative effect of progestin on endometrial cancer cells.

INTRODUCTION

Patients with advanced endometrial adenocarcinoma are usually treated with progestins or cytotoxic chemotherapy. Most clinicians prefer to initiate treatment with progestins because of the lesser toxicity, although only one-third of the patients with endometrial cancer respond objectively to the progestin therapy (1-3). This approach, however, results in an undesirable delay in instituting alternate therapy in many patients. Any method that predicts hormonal insensitivity of endometrial carcinoma would be helpful in selecting these patients for chemotherapy without delay. According to the current concept of steroid hormone action, hormonal effects are mediated through steroid receptors. Recent studies have shown that breast tumors having both ER and PgR are likely to respond to endocrine therapy, with the response rate approaching 80% and that when both receptors are absent, remissions occur in less than 10% of the cases (4). In endometrial cancer, therefore, responsiveness to progestins is expected to depend on the presence of PgR. However, only a few studies with a limited number of patients with endometrial adenocarcinoma have demonstrated a positive correlation between the presence of PgR and clinical response to progestin therapy (5-7). Furthermore, the basic mechanism by which progestins produce regression of endometrial cancer has not been fully established. A human endometrial cancer cell line, Ishikawa cells, having both ER and PgR was recently established (8) by one of us (M. Nishida, University of Tsukuba, Ibaraki, Japan). Upon receipt in our laboratory, these cells have been maintained for over 50 passage generations. We have found that a subline of Ishikawa cells (IK-90) in our laboratory is anomalous since the cells contain PgR but not ER. IK-90 cells may, therefore, be an ideal model for studying the mechanism of regression of human endometrial cancer by progestins. In this paper, the characteristics of PgR and biochemical effects of progestins on IK-90 cells in culture are reported.

MATERIALS AND METHODS

Cell Culture. The original cell line, Ishikawa cells, was established in 1980 from explants of well differentiated tubular adenocarcinoma obtained from a 39-year-old woman and sustained a stationary period of 110 culture days (8). The cells have been found to form a monolayer in a mosaic fashion and to have a tendency to pile up. These cells (passage number 64) supplied in 1983 have been kept in continuous culture in our laboratory. They were routinely maintained in 75-cm² Falcon plastic flasks at 37°C containing Eagle's minimum essential medium (Handai biken, Osaka, Japan) supplemented with 10% FCS (Grand Island Biological Co., Grand Island, NY), 200 units penicillin, and 200 µg streptomycin/ml in a 5% CO2:95% air-humidified incubator. The cells were passaged biweekly using 0.25% trypsin in phosphate-buffered saline without Ca2+ and Mg2+ (Handai biken). The cells used for the present experiments were grown in medium containing charcoal-treated 5% FCS. Endogenous steroids in FCS were removed by incubating 100 ml serum with 0.25 g DCC pellet at 45°C for 30 min, as described by Horwitz et al. (9). The pellet was prepared using DCC solution (0.25% charcoal and 0.0025% dextran in 0.01 M Tris-HCl, pH 8.0 (Sigma Chemical Co., St. Louis, MO). Steroids dissolved in ethanol were added to culture medium at a final concentration of less than 0.1% ethanol. In the present study, IK-90 cells (passages 80-105), a variant subline of Ishikawa cells harvested in our laboratory were used. The original cells (passages 28-34) maintained by Dr. M. Nishida, University of Tsukuba, were also utilized in some experiments.

Subcellular Fractions. All procedures were carried out at 0-4°C. Confluent cells (approximately 1.0 × 10^6/dish) in Falcon plastic dishes (100 x 20 mm) were washed once with HBSS, pH 7.2 (Handai biken), and removed by scraping with a rubber policeman in 2 ml buffer containing 0.32 M sucrose and 5 mM sodium phosphate, pH 7.4. For each determination, cells in 4 dishes were collected in a 15-ml tube. The collected cells were centrifuged at 150 × g for 5 min. The precipitated cells were resuspended in 2 ml of phosphate buffer (5 mM sodium phosphate, 5 mM mercaptoethanol, 3 mM MgCl₂, 10 mM sodium molybdate, and 20% glycerol, pH 8.0) and homogenized in a Teflon-glass homogenizer (approximately 30 strokes) until they were completely disrupted as seen by a phase contrast microscopy. The homogenate was first centrifuged at 800 × g for 10 min to obtain crude nuclei, and the supernatant was then centrifuged in a Hitachi RPS 65 rotor (Hitachi Sales Corp., Tokyo, Japan) at 105,000 × g for 60 min to obtain cytosol (1.0-1.4 mg protein/ml). The nuclear pellet (0.3-0.4 mg DNA) from the initial 800 × g centrifugation was resuspended in 1 ml of the phosphate buffer containing 0.6 M KCl and incubated for 1 h.

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2 The abbreviations used are: ER, estrogen receptors; PgR, progesterone receptors; R5020, promegestone(17,21-dimethyl-19-nor-4,9-pregna-2,11-diene-3,20-dione); FCS, fetal calf serum; HBSS, Hank's balanced salt solution; DCC, dextran-coated charcoal.
proteins from the nuclear extract were then obtained by centrifugation. The nuclear extract was diluted 6-fold to reduce the salt concentration, a step essential for DCC assay (9).

ER and PgR Assays. Triplicate aliquots of cytosol (0.3 ml) or diluted nuclear extract (0.5 ml) were incubated with various concentrations (0.6–15 nM) of either 17β-[3H]estradiol (100 Ci/mmol; New England Nuclear, Boston, MA) or 3H-labeled R5020 (77 Ci/mmol; New England Nuclear) at 0–4°C for 16 h. On some occasions, the incubation was carried out at 25°C for 16 h. Nonspecific binding was determined by parallel incubations with either 17β-[3H]estradiol or 3H-labeled R5020 in the presence of a 200-fold m excess of diethylstilbestrol or R5020. For competitive binding study, cytosols (0.3 ml) were incubated with 10 nM 3H-labeled R5020 in the presence or absence of various concentrations of several steroids (5 nM–50 μM) at 0–4°C for 16 h. After incubation, 30 μl (for cytosol) or 50 μl (for nuclear extract) of 2.5% charcoal-0.025% dextran solution were added, and the mixtures were incubated at 0–4°C for 15 min. The charcoal was removed by centrifugation, and 0.25-ml (for cytosol) or 0.45-ml (for nuclear extract) aliquots of the supernatant were counted. In preliminary experiments, the DCC assay was found to be useful for the estimation of tritium bound to macromolecules in the diluted nuclear extracts, since the specific binding sites per μg protein obtained by the DCC assay was identical to those obtained by gel filtration (passing 0.2 ml of the mixture through a 1 × 18-cm Sephadex G-25 column; Pharmacia Fine Chemicals, Uppsala, Sweden).

For detection of ER, a whole cell labeling assay was also performed as described by Fleming et al. (10), unless specified otherwise. Cells in the dishes were incubated with 10 nM 17β-[3H]estradiol with or without 2 μM diethylstilbestrol in medium containing 20 mM sodium molybdate, at 0–4°C for 22 h. After washing with HBSS, the incubated cells were removed by scraping and homogenized in 2 ml buffer (10 mM Tris-HCl, 1 mM MgCl₂, 0.1 mM dithiothreitol, 20 mM sodium molybdate, 30% glycerol, and 0.05% Triton X-100, pH 8.0). The homogenate was centrifuged at 150 × g for 5 min, and then the supernatant was incubated with 0.2 ml of 2.5% DCC solution at 0–4°C for 15 min. Aliquots (0.5 ml) of the supernatant were counted.

Sucrose Density Gradient Centrifugation. Linear 5–20% sucrose density gradients (4.5 ml) were prepared in the phosphate buffer. The aliquots (0.3 ml) of cytosols (1.6–2.4 mg protein/ml) were incubated with 10 nM of either 17β-[3H]estradiol or 3H-labeled R5020 at 0–4°C for 4 h. The tritium bound to macromolecules was separated from nonbound tritium by the DCC method, and 0.25 ml of the supernatants was applied to the top of the gradients, followed by centrifugation in a Hitachi RPS 50 rotor at 216,000 × g for 14 hr at 0–4°C. Fluorescent bovine serum albumin (4.65S) and human γ-globulin (7.0S) were used as internal markers (11). After centrifugation, 0.2-ml fractions were collected into tubes and counted.

PgR Induction by 17β-Estradiol. Cells were grown in culture dishes for 5 days and then medium was replaced by medium containing 10 nM 17β-estradiol. The cells were further grown at 37°C for the times indicated in the text. Fluid in the dishes were changed every 48 h. At the end of incubation, the cells were washed with ice-cold 5 ml of medium 3 times to remove 17β-estradiol. The cells were then homogenized in 2 ml of the phosphate buffer as described above, and cytoplasmic PgR was estimated with a single saturating dose, 10 nM 3H-labeled R5020, followed by DCC assay.

PgR Translocation. One mM progesterone in 5 μl ethanol was added to 5 ml medium containing confluent cells in culture dishes, and the cells were incubated at 37°C for 1 h. At the end of incubation, the cells were immediately washed 3 times with 5 ml of ice-cold medium to remove progesterone. After washing, the cells in dishes were kept at 37°C for the periods shown in the text. The cells were then washed and homogenized in 2 ml of the phosphate buffer, and cytoplasmic and nuclear PgR were measured with 10 nM 3H-labeled R5020, followed by DCC assay.

Effects of Steroids on Cell Growth. In the preliminary experiments, IK-90 cells were found to grow substantially in medium containing 1% DCC-treated FCS, although the growth rate in 1% FCS was slower than in 5 or 10% FCS. Since it has been reported that sensitivity of human mammary cancer cells to steroid hormones is reduced as the concentration of serum in medium is increased (12, 13), the effects of steroids on cell growth were examined at the concentration of 1% FCS. Cells (4 × 10⁶/dish) were plated in Falcon plastic dishes (60 × 15 mm), and 2 days after plating, medium in triplicate dishes was replaced by medium containing several steroid hormones at various concentrations. Fluid in the dishes was changed every 48 h. The treated cells were removed by trypsinization at the times indicated in the text, and a number of cells per dish were counted by hemocytometer.

Periodic Acid-Schiff Staining. Cells (1 × 10⁶/chamber) were plated in tissue culture chamber slides (Lab-Tek Products, Naperville IL), and 2 days after plating, medium in chamber slides was replaced by medium containing R5020 at various concentrations. Three days after starting incubation with R5020, medium was removed and cells were fixed in 5% polyethylene glycol and 64% isopropanol alcohol and stained with periodic acid-Schiff for glycogen.

Assay of 17β-Estradiol Dehydrogenase. To study the effect of progesterin on the levels of 17β-estradiol dehydrogenase, subconfluent cells in culture dishes were incubated with either 10 nM or 1 mM R5020 for 5 days. At the end of incubation, the cells were washed with HBSS and homogenized at 0–4°C in a glass homogenizer with 50 mM Tris containing 1.4 mM NAD⁺, pH 8.0. The homogenates were centrifuged at 800 × g for 10 min. The supernatants (2.8–3.5 mg protein/ml) were processed for the determination of 17β-estradiol dehydrogenase activity. The assay was performed as described by Satyaswaroop et al. (14). 17β-Estradiol dehydrogenase activity was expressed as nmol of estrone formed from 17β-estradiol per mg protein per h.

Chromosomal Analysis. A preparation for chromosome analysis was carried out as reported by Kuramoto et al. (15). More than 100 metaphase cells were observed in order to calculate the modal ploidy, and 50 of the modal range were counted exactly to evaluate the chromosomal number. The karyotype of these was strictly analyzed in accordance with Denver nomenclature.

Miscellaneous Assays. The protein concentration of cytosol was measured by the method of Lowry et al. (16) using bovine serum albumin as standard. The DNA content of crude nuclear pellet was determined by the diphenylamine method (17). The radioactivity for tritium was assayed in 5 ml scintillation fluid (100 mg POPOP-3 g 2,5-diphenyloxazole in 666 ml toluene-333 ml Triton X-100) at an efficiency of 45% in an Isocap liquid scintillation counter.

Statistics. All values were expressed as mean ± SE. Student’s unpaired t test was used for the statistical analysis.

RESULTS

ER and PgR. As shown in Fig. 1A, saturation analysis of cytoplasmic progesterin binding sites in IK-90 cells revealed a...
curve for specifically bound 3H-labeled R5020. Scatchard plot analysis (18) showed that an apparent $K_d$ for R5020 was 1.0 nM with maximum binding sites of 158 fmol/mg protein (Fig. 1B). Since R5020 has been shown to bind to glucocorticoid receptors as well as to PgR, the findings shown in Fig. 1 were obtained in the presence of a 200-fold m excess of cortisol. These values were identical with those obtained in the original Ishikawa cells and are also close to those reported previously in human endometrial adenocarcinoma (19, 20).

In contrast to PgR, saturable binding sites for 17β-[3H]-estradiol were not observed in either cytosol or nuclear extracts of IK-90 cells incubated at 0–4°C (Fig. 2). Specific binding sites for 17β-estradiol were also undetectable in the crude nuclear pellets. Since some estrogen-filled nuclear sites could be demonstrated in variant human breast cancer cells (9), the incubation was also performed at 25°C for 16 h. Again, saturable binding sites for 17β-estradiol could not be observed in either nuclear or cytosol fraction. Furthermore, a whole cell labeling method was next conducted. Again, measurable or significant amounts of specific binding sites for 17β-estradiol were not observed. On the other hand, saturable binding sites for 17β-[3H]estradiol ($K_d$, 2.5 nM; maximum binding sites, 21.1 fmol/mg protein) were present in the cytosol of the original Ishikawa cells, although the affinity and the number of binding sites were relatively low.

The competitive binding assay in IK-90 cells indicated a binding specificity for progestins (Fig. 3), which is characteristic of PgR found in human endometrial adenocarcinoma (21) and in other progestin-sensitive tissues (22–24). The synthetic progestin, R5020, was the most potent competitor of 3H-labeled R5020 binding in the cells, which was followed by progesterone and 5α-pregnane-3,20-dione. Deoxycorticosterone, 17α-hydroxyprogesterone, and cortisol did compete, but not effectively. Neither testosterone nor 17β-estradiol bound to 3H-labeled R5020 binding sites in these cells.

Sucrose density gradient analysis under the low-salt condition was carried out in IK-90 cells to examine the 3H-labeled R5020 binding sites. As shown in Fig. 4, the major radioactive peak of cytoplasmic progestin binding molecules was found at the 4S region with a lesser but a significant peak at the 8S area in both the presence and absence of excess cortisol. Similar sedimentation constants were observed in human endometrial cancer (5, 6, 21) and in other progestin target tissues (22, 24). Thus, the addition of a 200-fold excess of cortisol in the reaction mixture resulted in only slight alterations of specific 3H-labeled R5020 values. DOC, deoxycorticosterone; 17α-OHP, 17α-hydroxyprogesterone; $E_A$, 17β-estradiol.

Fig. 3. Competition of various steroids for 3H-labeled R5020 binding in IK-90 cells. Aliquots of the cytosol (1.0–1.4 mg protein/ml) were incubated with 10 nM 3H-labeled R5020 in the presence or absence of various concentrations of steroids (5 nM–50 μM) at 0–4°C for 16 h. The bound 3H-labeled R5020 values are the means of triplicate determinations. Binding is expressed as the percentage of 3H-labeled R5020 bound in the absence of competitor: DOC, deoxycorticosterone; 17α-OHP, 17α-hydroxyprogesterone; $E_A$, 17β-estradiol.
90 cells were next treated with 17β-estradiol for 7 days. However, 7-days' treatment with 17β-estradiol did not significantly enhance the PgR level (64.9 ± 10.5 fmol/10⁷ cells). In contrast to IK-90 cells, a significant increase in PgR was observed in the original cells by addition of 10 nm 17β-estradiol for 5 days (67.2 ± 10.2 and 146.4 ± 15.0 fmol/10⁷ cells in the absence and presence of 17β-estradiol, respectively), indicating that the original Ishikawa cells possess functional ER.

PgR observed in IK-90 cells appear to be typical PgR by several criteria; the receptors bind only progestins with high affinity, and they sediment at both 8S and 4S regions on low-salt sucrose gradients, although they are not enhanced by estrogen. We next studied whether the PgR are regulated by progestins. When IK-90 cells were treated with 1 μM of progesterone for 1 h, a rapid and significant increase in nuclear PgR with a concomitant decrease in cytoplasmic PgR was observed (Fig. 5). However, the nuclear receptor levels returned to the pretreatment level 1 h after progesterone removal, although the cytoplasmic receptors still depleted. Total receptor levels therefore fell rapidly; about 50% of the sites were lost in 1 h. Four h after the progesterone removal, cytoplasmic PgR gradually increased and were fully replenished 24 h after the removal. These results suggest that PgR in IK-90 cells are apparently controlled by progestin.

Effect of Progestins on Cell Growth. Fig. 6 shows that the doubling time of log-phase IK-90 cells in medium containing 1% DCC-treated FCS was 48 h. Addition of various concentrations of R5020 reduced the cell growth in a dose-dependent manner. The doubling time was approximately 58 and 85 h in the presence of 0.1 and 1 μM of R5020, respectively. As a result, significant inhibitory effects on the cell growth were observed 3 and 7 days after the addition of 1 and 0.1 μM of R5020, respectively. Although the growth of cells was markedly reduced by the presence of a higher concentration of R5020, the cells survived during the period of study. The growth-inhibitory effects of progesterone on IK-90 cells were also examined under similar conditions; the inhibition in the presence of progesterone was lower than that in the presence of R5020 (Fig. 7). This effect can be explained by the following reasons: (a) progesterone has been reported to be rapidly (Δt, 2–4 h) metabolized in culture medium containing human endometrial cancer cells (14); (b) R5020 seems to have a higher affinity for PgR than for progesterone in these cells (Fig. 3). In the next experiment, the effects of other classes of steroids on the growth of IK-90 cells were examined. Cortisol, testosterone, 17β-estradiol, or tamoxifen was found to have no effect on the growth of the cells at the concentrations (1 nm–1 μM) examined (data not shown). These results indicate that IK-90 cells are estrogen insensitive, and the growth-inhibitory effect of progestins on IK-90 cells is specific; the effect of progestins is mediated through PgR but not through glucocorticoid receptors.

Periodic Acid-Schiff Staining. Fig. 8 shows photomicrographs of a sample of IK-90 cells before and after incubation with R5020. No glycogen vacuoles were observed in the control cells to which progesterin was not added (Fig. 8A). Although the appearance of glycogen deposit in cytoplasm was faint in the presence of 10 nm R5020, 0.1 μM R5020 evidently produced periodic acid-Schiff-positive granules in an enlarged cytoplasm (Fig. 8B), which was sensitive to diastase treatment. The intensity and number of granules were more pronounced in the presence of 1 μM R5020. These results indicate that progesterin...
caused an accumulation of glycogen in the cytoplasm of IK-90 cells.

Assay of 17β-Estradiol Dehydrogenase. The activity of 17β-estradiol dehydrogenase in IK-90 cells was found to be very low (0.21 nmol estrone formed/mg protein/h). The enzyme activities did not increase significantly in the cells cultured for 5 days in the presence of 10 nM and 1 μM R5020 (0.23 and 0.28 nmol estrone formed/mg protein/h, respectively). R5020 thus failed to increase the activity of 17β-estradiol dehydrogenase, a progesterin-sensitive enzyme, in IK-90 cells. There have been no instances, however, in which a significant increase in 17β-estradiol dehydrogenase in response to progestins can be demonstrated in endometrial carcinomas in culture (29).

Chromosome Analysis. Chromosome analysis was performed on both the original cells (28th generation) and IK-90 cells (90th generation). A majority of the metaphase cells in either cell line was apparently found at the diploid range. An exact count of this modal range was done for further evaluation; the modal chromosome number was 46 (90%) in the original cells. On the other hand, the modal chromosome numbers, 51 and 52 (50%) were predominant over 46 (40%) in IK-90 cells. A marker chromosome, der (11)(t(4q;11q), was observed in both the original and IK-90 cells by karyological analysis.

DISCUSSION

The present results demonstrate that progestin has an inhibitory effect on the growth of IK-90 cells containing PgR, a variant subline of the original Ishikawa cells derived from human endometrial adenocarcinoma. Currently, there are few model systems available for studying the mechanism of hormonal regulation of human endometrial cancer. Since the establishment of the human endometrial cancer cell line HEC-1 (15), several cell lines have been established (30, 31). However, they lack PgR and do not respond to progestins. To our knowledge, the present results show for the first time the presence of a progestin-responsive human endometrial cancer cell line that contains estrogen-independent functional PgR. It was shown that the levels of cytoplasmic PgR necessary to elicit progestational effects in human endometrial cancer appear to be at or above 200 fmol/mg protein (32, 33). Although the PgR level found in IK-90 cells in the present study (158 fmol/mg protein) was slightly lower than the lower limit reported previously (32, 33), the level seems to be able to induce progestational effects in IK-90 cells.

Satyaswaroop and Mortel (34) have reported that the lack of progestin sensitivity in cultured endometrial carcinoma explants is due to PgR instability under culture conditions. However, IK-90 cells kept in our continuous culture have been found to contain stable and functional PgR. Biochemical studies have shown that characteristics of PgR in IK-90 cells appear to be the same as those observed in typical PgR. A rapid turnover of the receptors inside the cells, processing state, and replenishment of the receptors following progestin treatment were also observed. Similar observations have been reported in studies of human breast cancer MCF-7 (23) and T47D cells (9) and in mammalian uteri (35, 36).

On the other hand, estrogen-binding sites were absent in IK-90 cells. In addition, 17β-estradiol did not increase the levels of PgR. Furthermore, neither 17β-estradiol nor antiestrogen affected the growth of IK-90 cells. It can be concluded therefore that IK-90 cells are ER negative and estrogen insensitive. It is generally accepted that estrogen, presumably acting through ER, is required for PgR induction in estrogen and progestin target tissues. The cells we describe here are anomalous. Since estrogen had no significant effect on PgR levels in IK-90 cells without ER, PgR in these cells are not controlled by estrogen. In fact, a small group of anomalous endometrial adenocarcinomas exist that have no ER yet contain PgR (27, 37, 38). We also demonstrated that the original Ishikawa cells possess saturable binding sites for both estrogen and progesterin, and the PgR can be induced by estrogen. Recently, similar results were reported by Holinka et al. (39) using the original Ishikawa cells. The studies on chromosome analysis showed that there exists a difference in a modal chromosome number between the original and IK-90 cells although a marker chromosome is present in both cells. It may be considered therefore that some divergences occurred during continuous cultures of Ishikawa cells resulting in an appearance of IK-90 cells having estrogen-independent PgR, a subline of the original Ishikawa cells.
Inhibition of the growth of IK-90 cells was found to be progestin specific. It was reported that progestins suppress the growth of cells in culture and induce differentiation of previously established cells (30, 31) in which PgR was absent. However, the effective concentration of progestins (more than 10 μM) was pharmacological. In the present study, on the other hand, the inhibitory effect of progestin on the growth of IK-90 cells was dose dependent (Fig. 6), and 0.1 μM R5020 significantly suppressed the growth of cells. In addition, R5020 also caused accumulation of glycogen in cytoplasm of IK-90 cells.

These results demonstrate that IK-90 cells are progestin sensitive. However, we failed to demonstrate a significant biological effect of progestins in IK-90 cells at concentrations compatible with the affinity constant for PgR. An establishment of a culture system in serum-free medium in which IK-90 cells can grow is under way in our laboratory, since some components present in serum are considered to reduce the effects of steroid hormones in culture.

At present, it is not well known whether progestins inhibit the growth of endometrial adenocarcinoma cells directly or indirectly through other hormones. Progestins have been shown to exhibit antioestrogenic actions in several estrogen-sensitive tissues. However, the present results regarding PgR-positive but ER-negative IK-90 cells strongly suggest that the antiproliferative effects of progestins on these cells are mediated directly through PgR but not through antioestrogenic action of progestins. On one hand, Horwitz and Freidenberg (40) have recently demonstrated that the antitumor effects of progestins on breast cancer cells in culture are achieved via the PgR system by mechanisms different from those of antioestrogens. From the viewpoint obtained in the present studies, it may be concluded that progestins can produce regression of endometrial cancer directly through the PgR system.

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