Estrogen Stimulation and Tamoxifen Inhibition of Leiomyoma Cell Growth *in Vitro and in Vivo*

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ABSTRACT

Uterine leiomyomas (fibroids) are the most common gynecological neoplasms and may be associated with significant morbidity. Recently, we described a rat model (Eker rat) of fibroid development in which reproductive tract leiomyomas develop spontaneously with high frequency. The present studies describe the estrogen and antiestrogen responsiveness of an Eker rat leiomyoma-derived cell line *in vitro* and *in vivo*. In this cell line, estradiol stimulated growth in estrogen-depleted medium, whereas the nonsteroidal antiestrogen tamoxifen maximally inhibited cell proliferation in medium containing 10% charcoal-stripped serum. Proliferation was also decreased by the biologically active tamoxifen metabolite 4-hydroxytamoxifen; the metabolite was more effective than the parent compound in exerting this growth inhibition. Compared to placebo-treated controls, estradiol increased the size of tumors that developed in a nude mouse xenograft system, whereas tamoxifen increased tumor latency and decreased tumor size. This study of leiomyoma cells in a well-defined system suggests that antiestrogens may prove efficacious in the treatment of this clinically important neoplasm. (*Endocrinology* 136: 4996–5003, 1995)

Uterine leiomyomas (fibroids) occur in 20–25% of all women during the decades of reproductive life (1). Although leiomyomas are considered benign, up to 50% of these tumors may be associated with considerable morbidity (1). The clinical significance of these tumors is reflected in the facts that adequate medicinal agents are not currently available for myoma therapy, and 200,000 women annually undergo hysterectomy for this condition (2). Due to the high incidence, detrimental biological effects, and lack of satisfactory nonsurgical treatment regimens for uterine leiomyomas, these tumors have a substantial impact on the health of large numbers of women.

Despite the startling frequency at which leiomyomas occur, little information is available on their etiology, and the responses of these lesions to steroid hormones have not been examined in any systematic fashion. Clinical data, however, suggest that leiomyomas respond to steroid hormones. Fibroids are typically diagnosed during the decades of reproductive life, may increase in size during pregnancy, and often decrease in size after menopause (1). The effectiveness of GnRH agonists in shrinking these tumors also argues that their growth may be modulated by steroid hormones (3–5). Because of the temporal association of tumor growth with the reproductive years and pregnancy, estrogen has received much attention as a possible mediator of leiomyoma development.

The triphenylethylen antiestrogen tamoxifen has been used extensively in the treatment of clinical breast cancer (6). Although tamoxifen administration has been associated with possible increased risk of endometrial carcinoma (7), little information is available on the effects of this compound on myometrial growth and proliferation. Responses to tamoxifen are species and tissue specific (8), and the effects of tamoxifen on leiomyomas as a group have yet to be elucidated. This fact becomes especially important because of the high incidence of leiomyoma in the general population. Tamoxifen is currently being evaluated as a preventative for breast cancer (6), and the effects of this compound on the female population, which has a high background incidence of myometrial tumors, should be considered.

Systematic studies on the roles of hormones and antihormones in leiomyoma development have been hampered because no animal model for the human condition has been previously available. However, we recently described the novel Eker rat model in which reproductive tract leiomyomas arise spontaneously with high frequency (9, 10). These tumors arise as part of a hereditary cancer syndrome resulting from a germline mutation in what has recently been identified as the tuberous sclerosis 2 gene (11–14). In this model system, we evaluated the responses of rat leiomyoma to estrogen, the antiestrogen tamoxifen, and its biologically active (15) metabolite 4-hydroxytamoxifen. Cell proliferation was used to evaluate the *in vitro* responses of a leiomyoma-derived cell line to these compounds. In addition, the ability of estradiol and tamoxifen to modulate the *in vivo* growth of leiomyoma cells in a nude mouse xenograft assay was de-
Materials and Methods

Cell culture

The Eker rat uterine leiomyoma-derived cell line ELT 3 was maintained and propagated in medium as previously described (9). ELT 3 cells were grown on plastic (Corning, Corning, NY) in a medium consisting of Ham's F-12-Dulbecco's Modified Eagle's Medium (DMEM; Gibco: BRL, Grand Island, NY) in equal amounts with 1.6 x 10^-6 M ferrous sulfate (Sigma Chemical Co., St. Louis, MO), 1.2 x 10^-8 U/ml vasopressin (Sigma), 1.0 x 10^-8 M T3 (Sigma), 0.025 mg/ml insulin (Sigma), 1.0 x 10^-6 M cholesterol (Sigma), 2.0 x 10^-7 M hydrocortisone (Sigma), and 10 pg/ml transferrin (Sigma) supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT; termed DF8 medium) and 50% 3T3 fibroblast-conditioned medium. The 3T3-conditioned medium was obtained by plating Swiss 3T3 cells at 25% confluence in DMEM supplemented with 10% calf serum (Colorado Serum Co., Denver, CO). After 3-4 days, this medium was harvested and filtered through a 0.45-μm cellulose acetate filter (Corning). The final medium preparation of DF8 and 3T3-conditioned media was termed 50:50. The cultures were maintained in a humidified 5% CO2 atmosphere at 37°C and subcultured every 4-7 days with 0.05% trypsin-0.02% EDTA (Gibco) in PBS (Gibco). Variations of the 50:50 propagative medium used to evaluate estrogen and antiestrogen responsiveness are indicated in the sections below.

Estrogen responsiveness of ELT 3 cells

The estrogen responsiveness of ELT 3 cells was measured by plating cells in either 50:50 or DF8 medium into 24-well plastic tissue culture dishes (Corning) at 10^4 cells/well. Cells were allowed to plate for 24 h. They were then rinsed with sterile PBS and refed phenol red-free DF8 (using phenol red-free DMEM and F-12; Gibco) containing 10% charcoal-stripped FBS, verified by the manufacturer to contain less than 5.0 mg/ml 17β-estradiol (Sigma), 1.0 x 10^-5 U/ml vasoressin (Sigma), 1.0 x 10^-5 M cholesterol (Sigma), 2.0 x 10^-7 M hydrocortisone (Sigma), and 10 pg/ml transferrin (Sigma) supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT; termed DF8 medium) and 50% 3T3 fibroblast-conditioned medium. The 3T3-conditioned medium was obtained by plating Swiss 3T3 cells at 25% confluence in DMEM supplemented with 10% calf serum (Colorado Serum Co., Denver, CO). After 3-4 days, this medium was harvested and filtered through a 0.45-μm cellulose acetate filter (Corning). The final medium preparation of DF8 and 3T3-conditioned media was termed 50:50. The cultures were maintained in a humidified 5% CO2 atmosphere at 37°C and subcultured every 4-7 days with 0.05% trypsin-0.02% EDTA (Gibco) in PBS (Gibco). Variations of the 50:50 propagative medium used to evaluate estrogen and antiestrogen responsiveness are indicated in the sections below.

Determination of experimental conditions for maximum in vitro tamoxifen responsiveness

Individual breast carcinoma cell lines have been shown to vary in their sensitivity to tamoxifen. A pilot experiment was, therefore, conducted to determine the medium in which tamoxifen's effects on leiomyoma cells would be maximal. Tamoxifen responsiveness in ELT 3 was measured by plating cells in DF8 medium into 24-well plastic tissue culture dishes. Cells were allowed to proliferate for 72 h and then rinsed with sterile PBS. Individual wells were next reseeded with DF8 containing tamoxifen citrate (Sigma) at concentrations from 0.1-10 μM and 10% FBS, 10% charcoal-stripped FBS, 25% FBS, or 25% charcoal-stripped FBS; 0.1% ethanol as a vehicle control was also added to each medium type. Cells in triplicate wells were resuspended by trypsinization and counted with a Coulter counter at 24-h intervals. In each well, the medium/treatment compound was changed after 72 h of exposure. Inhibition of proliferation was determined by expressing cell number in treatment groups as a percentage of cell numbers in the appropriate control medium and comparing values.

Tamoxifen responsiveness in ELT 3 cells

The tamoxifen responsiveness of leiomyoma cells was measured by plating ELT 3 in DF8 medium into 24-well plastic tissue culture dishes. Cells were allowed to proliferate for 24-72 h. They were then rinsed with sterile PBS and refed DF8 containing 10% charcoal-stripped FBS plus 0.1% ethanol (vehicle control) or tamoxifen citrate (Sigma) at concentrations of 0.1-10 μM. Cells were then rinsed with sterile PBS and refed DF8 containing 10% charcoal-stripped FBS plus 0.1% ethanol (vehicle control) or tamoxifen at concentrations of 0.1, 1.0, and 5.0 μM. After 72 h, fresh medium containing ethanol or tamoxifen was added to the appropriate wells. In addition, in some wells previously containing tamoxifen, tamoxifen-containing medium was replaced with medium containing tamoxifen plus 17β-estradiol at a concentration one tenth that of the tamoxifen concentration. Cells in triplicate wells were resuspended by trypsinization and counted with a Coulter counter at 24 h intervals.

4-Hydroxytamoxifen responsiveness in ELT 3 cells

The ability of the biologically active metabolite 4-hydroxytamoxifen to modulate estrogen receptor activity was assessed by plating cells in DMEM supplemented with 10% charcoal-stripped FBS; Hyclone Laboratories, Logan, UT; termed DF8 medium) and 50% 3T3 fibroblast-conditioned medium. The 3T3-conditioned medium was obtained by plating Swiss 3T3 cells at 25% confluence in DMEM supplemented with 10% calf serum (Colorado Serum Co., Denver, CO). After 3-4 days, this medium was harvested and filtered through a 0.45-μm cellulose acetate filter (Corning). The final medium preparation of DF8 and 3T3-conditioned media was termed 50:50. The cultures were maintained in a humidified 5% CO2 atmosphere at 37°C and subcultured every 4-7 days with 0.05% trypsin-0.02% EDTA (Gibco) in PBS (Gibco). Variations of the 50:50 propagative medium used to evaluate estrogen and antiestrogen responsiveness are indicated in the sections below.

Modulation of ELT 3 tumor growth in nude mouse xenograft system

ELT 3 cells have been previously shown to produce tumors with a short latency when injected into athymic nude mice (9). The ability of 17β-estradiol and tamoxifen to modulate the growth of tumors in this nude mouse xenograft system was determined by inoculating 8- to 10-week-old intact female BALB/cAnNc-nu/nu mice (NCI, Frederick, MD) sc with 2 x 10^6 cells/site over each hip. Five to 9 days before inoculation, mice were anesthetized with methoxyflurane inhalation (Pitman-Moore, Mundelein, IL), separated into three treatment groups, and implanted with pellets of 17β-estradiol [one 1.7-mg 60-day release pellet in each mouse (n = 5); Innovative Research, Toledo, OH], tamoxifen free base [one 25-mg or five 5-mg 60-day release tablets in each mouse (n = 5); Innovative Research, or placebo pellets (n = 6); Innovative Research] sc in the interscapular area. Fresh pellets were implanted 8 weeks after initial implantation. Immediately before inoculation, cells were harvested during log phase growth and resuspended in 0.1-0.2 ml serum-free DF8 medium. Animals were observed weekly postinoculation, and tumor development was measured with a Vernier caliper. The mean cross-sectional area of each tumor was calculated as follows: (length/2) x (width/2) x π. Mice were killed by CO2 inhalation when tumors grew to greater than 1.5 cm in diameter or approximately 120 days after inoculation. A portion of each tumor was frozen at −70°C.
for subsequent analysis, and the remainder of the sample was fixed in 10% neutral buffered formalin for histopathological examination. Fixed tumor samples were embedded in paraffin by routine methods, sectioned, stained with hematoxylin and eosin, and examined under a microscope.

**Statistical analysis**

Differences between treatment groups in all experiments were evaluated using analysis of variance or Student’s unpaired *t* test (StatView, Abacus Concepts, Berkeley, CA). All data points are reported as the mean ± SEM.

**Results**

**In vitro estrogen and antiestrogen responses**

To determine whether the leiomyoma-derived cell line ELT 3 would respond to estrogen present in the growth medium, cells were grown in estrogen-depleted medium (phenol red-free medium and stripped serum) supplemented with 0.1 nM to 1.0 μM 17β-estradiol. Estrogen produced a dose-dependent increase in proliferation in the cell line (Fig. 1). The stimulation of cell growth was significant (*P* < 0.05) at all hormone concentrations examined. The maximal response was seen at the highest concentration (1.0 μM estradiol), which increased cell number approximately 2.2-fold over the control value after 96 h of continuous estrogen exposure. Passage of the cells in the absence of estrogen before plating did not affect the ability of the cells to proliferate in estrogen-depleted medium (data not shown). In addition, cells grown under these experimental conditions maintained their estrogen responsiveness. The fact that the estrogen receptor-negative leiomyoma cell line ELT 6 was not responsive to estradiol (data not shown) provides evidence that the effects of this hormone are specific and are not due to nonspecific stimulation of growth at higher concentrations.

Before embarking on large scale studies to examine the effects of the antiestrogen tamoxifen on cell line ELT 3, a pilot experiment was conducted to evaluate the effects of various serum types and concentrations on subsequent results. In the pilot experiment examining the effects of serum on tamoxifen responsiveness, the doubling times for cultures in all medium types were shortest on day 4 of the experiment (data not shown). Figure 2 illustrates the growth inhibition at this time point for each medium, expressed as a percentage of the appropriate control value. A comparison of the differing medium compositions revealed that, overall, tamoxifen exerted its greatest effects in 10% charcoal-stripped serum.

In 10% charcoal-stripped serum, the ability of tamoxifen to modulate the growth of leiomyoma cells was examined over a range of concentrations. Tamoxifen at concentrations of 0.1–10 μM produced a dose-dependent decrease in cell number that was significant at all concentrations tested. As shown in Fig. 3, after 4 days of tamoxifen exposure, a 34% decrease in cell number was seen at the lowest concentration (0.1 μM). Some experimental variation was seen in three independent experiments conducted to evaluate the tamoxifen response: for example, inhibition with 0.1 μM tamoxifen ranged from 20–34%. This inhibition increased with increasing tamoxifen concentrations, reaching 50%, 56%, 70%, and 77% at concentrations of 0.5, 1.0, 2.5, and 5.0 μM, respectively. Cells maintained in 10 μM tamoxifen appeared to slough off the dish at all time points examined and decreased in number throughout the course of the experiment. Some sloughing of cells was
Tumors in estrogen-treated animals were significantly larger than those in placebo-treated controls at each weekly time point. When tumor size was examined, however, latency was observed between 1/10 estradiol- and placebo-treated animals. Because tamoxifen is not extensively metabolized in vitro, the effect of 4-hydroxytamoxifen was assessed in leiomyoma cells and compared with that of tamoxifen. Treatment with the biologically active metabolite caused a dose-dependent decrease in cell number significantly more pronounced than that seen with tamoxifen under identical experimental conditions (P = 0.0098; Fig. 6). Cell numbers in wells containing 0.1 μM tamoxifen were not statistically different from numbers in wells containing 1.0 nM 4-hydroxytamoxifen, indicating that similar effects occurred at 100-fold differences in compound concentrations. In addition, 4-hydroxytamoxifen was effective at inhibiting proliferation at concentrations as low as 0.01 nM.

In vivo modulation of nude mouse tumor growth by estradiol and tamoxifen

Because the ELT 3 cell line is tumorigenic in nude mice (9), it was possible to examine the effects of estrogen and tamoxifen on tumor growth in vivo. No significant difference in tumor latency was observed between 17β-estradiol- and placebo-treated animals. When tumor size was examined, however, tumors in estrogen treated animals were significantly larger than those in placebo-treated controls at each weekly time point examined after 8 weeks postinoculation. For example, at week 13 postinoculation, placebo-treated animals had a mean tumor area of 0.985 ± 0.215 cm², whereas estrogen-treated mice had a mean tumor area of 1.885 ± 0.284 cm² (P = 0.0436; Fig. 7A).
FIG. 5. Evaluation of the ability of estrogen to rescue tamoxifen-induced growth inhibition of leiomyoma cells. Cells were exposed to tamoxifen (Tam) or 0.1% ethanol (Untreated) for 72 h. Fresh medium containing tamoxifen, tamoxifen plus 17β estradiol at one tenth the tamoxifen concentration, or ethanol was then added, and cell counts were made 48 h later. Data shown are expressed as a percentage of the cell numbers in tamoxifen treatment wells, which were assigned a value of 100%. Results are similar for both 0.1 μM (A) and 1.0 μM (B) tamoxifen concentrations, in which tamoxifen plus estradiol values (Tam+E2) were significantly greater than values in wells maintained continuously in tamoxifen. Tamoxifen plus estradiol values were also not significantly different from untreated values for these two tamoxifen concentrations. C, At a concentration of 5.0 μM tamoxifen, the addition of estradiol to the medium did not alter cell numbers significantly from values in wells maintained continuously in tamoxifen.

Discussion

Tumor latency in an ELT 3 nude mouse xenograft system was significantly increased in tamoxifen-treated animals vs. estrogen- or placebo-treated controls (P = 0.00033 and 0.00018, respectively; Fig. 7B). In addition, tumors in tamoxifen-treated mice were significantly smaller than those in placebo-treated animals. At the same week 13 point described above, placebo-treated animals had a mean tumor area of 0.985 ± 0.215 cm², and tamoxifen-treated mice showed a mean tumor area of 0.084 ± 0.022 cm² (P = 0.0243; Fig. 7A).

On histological examination, nude mouse tumors from all groups appeared similar. The neoplasms were comprised of solid sheets and interwoven fascicles of extremely anaplastic mesenchymal cells with extensive cellular pleomorphism. Masses from estrogen- and placebo-treated animals contained multiple coalescing regions of necrosis, which, in conjunction with the high mitotic index seen in these tumors, was indicative of rapid growth (Fig. 8). The masses from tamoxifen-treated animals were devoid of necrotic regions, indicating a slower proliferative rate in these lesions, consistent with the observed increase in tumor latency and decrease in tumor size observed for these lesions.

FIG. 6. 4-Hydroxytamoxifen responsiveness in the Eker rat leiomyoma-derived cell line ELT 3. Cells were counted after 96 h of exposure to the indicated concentrations of 4-hydroxytamoxifen or tamoxifen. These numbers demonstrated a dose-dependent decrease in proliferation in wells treated with varying concentrations of the metabolite. Cell numbers in 100 nM tamoxifen were not significantly different from numbers in 1.0 nM 4-hydroxytamoxifen. The data shown are representative of three independent experiments. *, Significantly different from the control at P < 0.05.
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Fig. 7. Modulation of nude mouse tumor formation by estradiol and tamoxifen. A, Mean tumor area of week 13 postinoculation tumors in nude mice treated with placebo, estradiol, or tamoxifen. Compared with the effects of placebo, estradiol caused an increase in tumor area, whereas tamoxifen caused a decrease. *, Statistically significant at \( P < 0.05 \). B, Latency of nude mouse tumor development in animals treated with placebo, estradiol, or tamoxifen pellets, shown as the proportion of injection sites remaining tumor free. Tumor latency was increased in tamoxifen-treated animals compared to that in estrogen- or placebo-treated controls (\( P < 0.05 \)).

available on the response of human leiomyoma lines to in vitro estrogen administration, the maximum 2.2-fold increase in proliferation observed in response to estrogen is similar to the almost 2-fold increase that has been reported in the estrogen-responsive MCF7 breast carcinoma cell line and the 2.2-fold increase in the human ovarian adenocarcinoma cell line PEO4 (20,21). In these cell lines, responses to estradiol were also seen at concentrations up to 1.0 \( \mu M \).

In vitro tamoxifen responsiveness of breast cancer cell lines has been shown to vary significantly with experimental conditions (16-18). The presence or absence of estrogen in serum as well as the serum concentration can affect the response of a single cell line to tamoxifen by up to 75-fold (16). These observations have led to the suggestion that experimental conditions should be stringently reported when examining the in vitro effects of tamoxifen. Previous studies indicating that increasing serum concentration protects cells against the inhibitory effects of tamoxifen have expressed results as absolute values (e.g. micrograms of DNA or cell number) rather than as a percentage of the control value (17, 18). The decrease in baseline proliferative rates seen with lesser serum concentrations could alone account for the lesser values reported for tamoxifen treatment in cells maintained in low serum in these experiments.

In a pilot study, we compared the effectiveness of tamoxifen at different concentrations in inhibiting proliferation by expressing our results as percentages of cell numbers in the appropriate control media. When we compared results in serum or charcoal-stripped serum at concentrations of 2.5% and 10%, leiomyoma cells maintained in 10% charcoal-stripped serum showed the greatest degree of growth inhibition by tamoxifen. Within this medium type, inhibition was greatest at tamoxifen concentrations of 0.1-2.5 \( \mu M \). Based on the results of our pilot study, we used medium containing 10% charcoal-stripped serum in all our subsequent tamoxifen and 4-hydroxytamoxifen experiments in vitro. Interestingly, in a report of doubling time as a percentage of the control value in T47D breast cancer cells treated with tamoxifen, the cells were most resistant to the effects of tamoxifen in 10% stripped serum (16). The reasons behind this
Tamoxifen is not thought to be rapidly metabolized or inactivated by cells in vitro. This thinking is supported by the fact that tamoxifen responsiveness is similar in cells left in tamoxifen-containing medium for a period of days and in cells in which medium containing tamoxifen is changed regularly (25). Because of the lack of metabolism of tamoxifen in vitro, we evaluated the response of leiomyoma cells to the biologically active metabolite 4-hydroxytamoxifen. This metabolite has been shown to be a more potent growth inhibitor than its parent and has been reported to be 50-167-fold more effective than tamoxifen in inhibiting the proliferation of MCF7 cells in vitro (25). Our experiments with leiomyoma cells yielded similar results; 4-hydroxytamoxifen was 100-fold more effective than tamoxifen in inhibiting cell growth.

Estrogen rescue experiments indicate that growth inhibition at tamoxifen concentrations of 1.0 \(\mu M\) or less is due to estrogen receptor-mediated mechanisms. The inhibition of proliferation seen with 5.0 \(\mu M\) tamoxifen, however, was not readily reversible by estrogen, suggesting that growth inhibition at higher concentrations occurs through a mechanism independent of the estrogen receptor. This phenomenon has also been described in several mammary carcinoma cell lines, in which tamoxifen concentrations greater than 1.0 \(\mu M\) are though to act via estrogen receptor-independent pathways (17). One possible caveat to this interpretation is that at higher tamoxifen concentrations, cells become irreversibly growth arrested or committed to growth arrest and, thus, cannot be rescued by estrogen. Data exist indicating that tamoxifen can induce apoptosis (26), and commitment to an apoptotic pathway could be an example of such an irreversible type of growth arrest. At present, the mechanism of growth arrest by tamoxifen in our system is not known, and additional experiments to examine the mechanism of growth inhibition by triphenylethylene in this model system are in progress.

The ability of ELT 3 to produce tumors with a short latency in athymic nude mice made possible the extension of in vitro data into an in vivo system. In a nude mouse xenograft assay, estradiol increased tumor size, but did not alter tumor latency compared with placebo treatments. Thus, it appears that estrogen does not alter the time course of events leading to tumor formation in the nude mouse system, but may increase the proliferative rates of tumors that do occur. This is similar to the observations in vitro, in which leiomyoma cells appear to be estrogen responsive rather than having an absolute requirement for estrogen. In addition, as intact female nude mice were used in these experiments, it is possible that the circulating levels of estrogen found in these animals were sufficient to support tumor formation in both estrogen- and placebo-treated mice.

Tamoxifen inhibited the latency of tumor formation in vivo, providing good correlation with in vitro data. Tamoxifen was not entirely effective in inhibiting tumor formation, however, as tumors did eventually arise in tamoxifen-treated animals. Histologically, these tumors appeared to be much slower growing than those arising in estrogen- or placebo-treated animals, as evidenced by the lack of significant necrosis. Cytostatic effects have been demonstrated in vivo in breast carcinomas grown in nude mice (27), and it may be that altered cell cycle transit times, similar to events reported in breast cancer cells in vitro (28, 29) may be the mechanism.
by which tamoxifen inhibits the growth of ELT 3 cells in the nude mouse system. In addition, tamoxifen resistance has been reported to occur in clinical breast cancer (30), and the possibility exists that the tumors that grew in tamoxifen-treated nude mice inoculated with leiomyoma cells were the clonal outgrowth of tamoxifen-nonresponsive cells.

Although tamoxifen responsiveness of myometrial tumors in women has not previously been examined experimentally, there are isolated reports of rapid increases in leiomyoma size in women taking tamoxifen (31, 32). The resulting tumors have not been well characterized, however, and it is possible that these isolated reports stem from the clonal expansion of a tamoxifen-resistant transformed myometrial cell. The likelihood of a resistant cell arising seems high because of the high tumor background in the large numbers of women taking tamoxifen. Tamoxifen is metabolized similarly in rats and humans (8), and future studies using Eker rats in which leiomyomas arise with a high spontaneous frequency will allow further characterization of the effects of tamoxifen on uterine tumors in situ.

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References


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