Presence of an Insulin-like Growth Factor I Autocrine Loop Predicts Uterine Fibroid Responsiveness to Tamoxifen

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ABSTRACT

Uterine leiomyoma is an estrogen-responsive tumor, and the present studies examine the ability of the antiestrogen tamoxifen to modulate leiomyoma cell growth. Tamoxifen is an effective form of hormonal therapy for breast cancer, although the mechanism by which tamoxifen inhibits tumor growth is not well understood and may involve mechanisms other than the action of tamoxifen as an estrogen antagonist. Tamoxifen was found to inhibit the proliferation of three of five leiomyoma-derived cell lines (ELT cell lines) in vitro, including an estrogen receptor-negative cell line. The ability of tamoxifen to decrease leiomyoma growth was found to correlate with expression of insulin-like growth factor I (IGF-I) by the tumor cells, suggesting that the inhibitory effects of tamoxifen were associated with expression of this growth factor. The existence of an IGF-I autocrine loop in the cells was investigated, because transcripts for both IGF-I and its cognate receptor were expressed in the tamoxifen-responsive cell lines. An IGF-I RIA demonstrated secreted IGF-I protein in serum-free medium conditioned by the IGF-I-expressing cell line ELT 3, and this same medium supported the growth of IGF requiring MCF-10A cells, indicating the presence of biologically active IGF-I in the conditioned medium. Exogenous IGF-I stimulated ELT 3 cell proliferation, confirming that this growth factor is mitogenic for leiomyoma cells. IGF-I neutralizing antibody inhibited ELT 3 growth, indicating that the levels of IGF-I produced by the leiomyoma cells were physiologically significant. These data demonstrate the existence of an IGF-I autocrine loop in tamoxifen-sensitive leiomyoma cells, supporting the hypothesis that the presence of an IGF-I autocrine loop predicts uterine fibroid responsiveness to tamoxifen.

INTRODUCTION

Gynecological leiomyomata in women consist of a spectrum of lesions ranging from benign myomas (fibroids) to highly malignant and metastatic leiomyosarcomas (1). These tumors are found in 20–25% of premenopausal women and arise from the smooth muscle cells of the uterine myometrium (1). Although leiomyomas are considered benign, they have a serious impact on the health of large numbers of women and may be associated with infertility, abortion, menorrhagia, dysmenorrhea, and postpartum hemorrhaging (2). Estrogen has received much attention as a possible mediator of leiomyoma growth, and numerous studies suggest a role for this hormone in the pathogenesis of tumor development in the myometrium (3–6).

It has been suggested that several growth factors, including EGF5 (7–9), TGFα (10), and PDGFs (11), may be involved in regulating proliferation in the uterus. In addition, IGF-I has been implicated as a potential mediator of estrogenic effects in this organ. IGF-I is a member of a larger family of peptides structurally related to insulin and exerts its effects through high-affinity binding to the transmembrane tyrosine kinase IGF-I receptor (12). IGF-I also binds with high affinity to a family of six soluble IGFBPs (13), and binding of the growth factor to these IGFBPs regulates the bioavailability of IGF-I to target cells (13). The widespread tissue distribution and temporal expression of IGF-I suggests that this growth factor may serve autocrine or paracrine functions (12). IGF-I appears to play an important role in the physiology of the normal myometrium. Utilizing in situ hybridization at the RNA level, IGF-I transcripts are detectable in all layers of the rat uterus but are most abundant in the longitudinal smooth muscle layer of the myometrium (14). IGF-I receptors have been localized in the uterus predominantly to the myometrial component (15), consistent with the hypothesis that IGF-I may act in an autocrine or paracrine manner in this tissue type.

Tamoxifen is a nonsteroidal antiestrogen used clinically as a mononocarinate salt (16). The pharmacology of tamoxifen and its effects on normal and neoplastic cells in vivo and in vitro have been studied extensively (16–18). Tamoxifen binds to the estrogen receptor and can competitively inhibit the binding of estradiol to its receptor (17). The therapeutic effects of this compound are not completely dependent on the presence of estrogen receptors in target cells. Although high levels of estrogen receptor in tumors are generally predictive of tamoxifen responsiveness (19), a lesser but still demonstrable effect is seen in estrogen receptor-negative breast cancers and other tumor types (20). It has been suggested that the presence of antiestrogen binding sites on tumor cells (21, 22), down-regulation of protein kinase C (23), and effects of the production of growth factors such as TGF-β and IGF-I by both target and stromal cells could contribute to growth inhibition of estrogen receptor-negative cells (20, 24, 25).

We have previously described a novel rat model in which reproductive tract leiomyomata arise spontaneously with high frequency (26, 27). These tumors arise as a result of the heritable transmission of a germline mutation in the tuberous sclerosis 2 gene (28–31). We have also previously described the establishment and characterization of leiomyoma-derived cell lines in this model (27). The present studies were designed to test the ability of tamoxifen to modulate the growth of a panel of five leiomyoma-derived cell lines and to examine the role of growth factors with the potential to mediate the action of tamoxifen inhibition of leiomyoma cell growth.

MATERIALS AND METHODS

Cell Culture. The Eker rat uterine leiomyoma-derived cell lines ELT 3, ELT 4, ELT 6, ELT 9, and ELT 10 (27) were maintained and propagated in DF8 medium, consisting of Hams F12-DMEM (Life Technologies, Inc., Grand Island, NY) in equal amounts with 1.6 × 10⁻⁷ M ferrous sulfate, 1.2 × 10⁻⁵ units/ml vasopressin, 1.0 × 10⁻⁴ M triiodothyronine, 0.025 mg/ml insulin, 1.0 × 10⁻⁴ M cholesterol, 2.0 × 10⁻⁷ M hydrocortisone, and 10 pg/ml transferrin (all from Sigma Chemical Co., St. Louis, MO) supplemented with 10% FBS (HyClone Laboratories, Logan, UT). Variations of the DF8 medium used to evaluate antiestrogen responsiveness and the existence of an IGF-I autocrine loop are indicated in the sections below. One of these cell lines, ELT

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The abbreviations used are: EGF, epidermal growth factor; TGF, transforming growth factor; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; FBS, fetal bovine serum.

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3, has been shown previously to be estrogen responsive and growth inhibited by tamoxifen (32). This cell line was used as a positive control for studies examining the effects of tamoxifen on the other leiomyoma-derived ELT cell lines.

**Tamoxifen Responsiveness in ELT Cell Lines.** The tamoxifen responsiveness of estrogen receptor-positive (27), leiomyoma-derived cell lines ELT 3, ELT 4, ELT 9, and ELT 10, as well as estrogen receptor-negative line ELT 6 (27), was measured by plating cells in DF8 medium into 12- or 24-well plastic tissue culture dishes (Corning Glass, Corning, NY). Cells were allowed to proliferate for 24—72 h until cells reached 10—40% confluence, depending on the doubling time of individual cell lines. Previous studies have shown that leiomyoma cells are most sensitive to the effects of tamoxifen when experiments are conducted in medium containing 10% charcoal-stripped serum (32). ELT cell lines were therefore rinsed with sterile PBS and refed with DF8 containing 10% charcoal-stripped FBS plus 0.1% ethanol (vehicle control) or tamoxifen citrate (Sigma Chemical Co.) at concentrations of 2.5—10 μM. Cells in triplicate wells were then resuspended by trypsinization and counted with a Coulter counter at the indicated intervals.

**4-Hydroxytamoxifen Responsiveness in ELT Cell Lines.** The ability of the biologically active metabolite 4-hydroxytamoxifen to inhibit the growth of estrogen receptor-positive (27) cell line ELT 10 was measured by plating cells in DF8 medium into 24-well plastic tissue culture dishes (Corning Glass). Cells were allowed to proliferate for 24 h. ELT 10 cells were then rinsed with sterile PBS and refed with DF8 containing 10% charcoal-stripped FBS plus 0.1% ethanol (vehicle control) or 4-hydroxytamoxifen (the kind gift of M. Gottardis) at concentrations of 0.01—100 μM. Cells in triplicate wells were then resuspended by trypsinization and counted with a Coulter counter at the indicated intervals.

**RNA Isolation and Northern Analysis.** RNA was extracted from log-phase cultures of ELT cell lines by the method of Chirgwin et al. (33). Polyadenylated RNA was isolated by oligodeoxythymidylate cellulose chromatography, separated by electrophoresis in 1.0% formaldehyde and agarose gels, and transferred to nitrocellulose by standard methods. Nitrocellulose filters were baked, prehybridized, and hybridized at 42°C for 48—72 h in 40% formamide and 3X SSC (1X SSC is composed of 0.15 M NaCl and 0.015 M sodium citrate) to complementary probes labeled with [3P]-dCTP to a specific activity of >1.0 × 10⁶ cpm/μg by random priming (Prime-It; Stratagene, La Jolla, CA). Probes used were prepared from cDNA inserts from the following plasmids: rat IGF-I (34), the kind gift of L. Murphy; rat IGF-I receptor (35) from American Type Culture Collection, Rockville, MD; murine EGF (pmegf10; Ref. 36), the kind gift of G. Bell; rat TGFα (Ref. 37), the kind gift of D. Lee; rat IGF II (38), the kind gift of M. Rechler; PDGF A (PDGF-α-D1; Ref. 39), the kind gift of C. Betscholtz; and PDGF B (v-sis; Oncor, Gaithersburg, MD; Ref. 40). For detection of IGF-I, IGF-I receptor, IGF II, and TGFα filters, were washed with four changes of 0.1X SSC and 0.05% SDS at 55—60°C for 2 h. For EGF, PDGF A, and PDGF B, filters were washed with four changes of 0.5X SSC and 0.1% SDS at 40°C for 2 h. All filters were then dried and exposed at −70°C to X-ray film with an intensifying screen. Lanes were normalized for differences in loading and RNA integrity by reprobing with glyceraldehyde-3-phosphate dehydrogenase (pGAD28) (41), the kind gift of F. Ferriola.

**Biological Assay for Functional IGF Activity.** Cells of the human mammary epithelial cell line MCF-10A were isolated from nonmalignant breast tissue and have an absolute requirement for IGFs; they proliferate in response to IGF-I in a dose-responsive manner in serum-free medium (42, 43). These cells were maintained on type I collagen-coated tissue culture dishes in serum-free Ham’s F12 medium supplemented with BSA, transferrin, ethanolamine, sodium selenite, triiodothyronine, insulin, hydrocortisone, and EGF (44). To test for IGF-like activity of conditioned medium from the ELT cell lines, DF8 medium devoid of serum and insulin and containing 1% BSA (basal medium) was collected from confluent cultures of ELT 3 cells after 48 h of incubation. Cell debris was spun out at 514 × g for 10 min. MCF-10A cells were seeded in 35-mm collagen-coated wells at 3.5 × 10⁵ cells per well in Ham’s F12 medium devoid of either insulin or EGF and containing 2.0% FBS to allow attachment. The next day, this plating medium was removed, and the cells were switched to the serum-free Ham’s F12 medium described above but without either insulin or EGF and with ELT 3-conditioned medium at 50% v/v. MCF-10A cells were subsequently grown for 7 days with two changes of medium, and the number of cells per dish was determined by counting isolated nuclei with a Coulter counter.

**IGF-I RIA.** ELT 3 cells were grown in basal medium as described above. Conditioned medium was harvested from the cells at the indicated intervals and quick frozen in liquid nitrogen. ELT 3-conditioned medium was then thawed and protease inhibitors (0.1 μg/ml phenylmethylsulfonyl fluoride and 2 μg/ml aprotinin) added. Medium was concentrated using a Filter Microse Protein Microconcentrator (MW cutoff, 1000; Filtron Technology Corp., Northborough, MA), at a relative centrifugal force of 7500 × g for 6 h at 4°C. After concentration, acetic acid was added to a final concentration of 0.1 M prior to storage at −80°C. Binding proteins were removed by extraction with SepPak C18 columns (Millipore, Bedford, MA). Columns were activated with 10 ml of methanol followed by 10 ml of water. To remove IGFBP's, 100 μl of thiolated, concentrated media were mixed with 900 μl of 1 M acetic acid and 5% BSA and loaded on the activated column. The sample was eluted by washing with 10 ml of 4% acetic acid, and IGF-I was eluted in 1.0 ml of 50% acetonitrile and 4% acetic acid. The sample was lyophilized and resuspended directly in 0.1 ml of RIA buffer. IGF-I RIA was performed using a rabbit antiserum and a second antibody to magnetic beads, according to the manufacturer’s instructions (Amersham, Arlington Heights, IL). IGF-I concentration in the conditioned medium was determined relative to a standard curve generated with known concentrations of IGF-I.

**IGF-I Mitogenicity in Leiomyoma Cells.** ELT 3 cells were plated in 24-well plastic tissue culture dishes in DF8 medium and allowed to attach and proliferate for 24—72 h. Wells were then washed twice with PBS, and medium was replaced with DF8 medium supplemented with BSA, insulin, and containing 0.5% charcoal-stripped serum (HyClone depleted medium). After 24 h, fresh depleted medium was added, containing either PBS (vehicle control) or human recombinant IGF-I (Collaborative Biomedical Products, Bedford, MA) at concentrations of 10—100 ng/ml. Cells in triplicate wells were resuspended by trypsinization and counted with a Coulter counter at the indicated intervals.

**IGF-I Neutralizing Antibody in Leiomyoma Cells.** ELT 3 cells were plated in 24-well plastic tissue culture dishes in DF8 medium and allowed to attach and proliferate for 48—72 h. Wells were then washed twice with PBS, and medium was replaced with basal medium. After 24 h, fresh basal medium was added, containing either mouse monoclonal antibody to human IGF-I (Upstate Biotechnology, Inc., Lake Placid, NY) at a concentration of 30 μg/ml or an isotype-matched mouse IgG1 (vehicle control; Sigma Chemical Co.). Cells in triplicate wells were resuspended by trypsinization and counted with a Coulter counter at the indicated intervals.

**RESULTS**

**Tamoxifen Responsiveness Correlates with IGF-I Expression.** The effect of tamoxifen on a panel of five Eker rat leiomyoma-derived cell lines (ELT lines) was examined over a range of concentrations. At high concentrations (10 μM), tamoxifen proved to be nonspecifically cytotoxic in all cell lines, causing significant vacuolization and sloughing of cells (data not shown). At lower concentrations, three of five leiomyoma cell lines (ELT 3, 4, and 9) exhibited similar growth inhibition in the presence of tamoxifen (Table 1). In these cell lines, cell number was reduced in a dose-dependent manner in cultures treated with tamoxifen. After 96 h of continuous exposure to 2.5 μM tamoxifen, for example, reduction in cell number versus control values was 16, 19, and 41% in ELT 4, ELT 9, and ELT 3, respectively. After similar exposure to 5.0 μM tamoxifen, reduction in cell number was 27, 32, and 59% in the same cell lines. Data from a representative experiment showing the in vitro responsiveness of ELT 4 to tamoxifen are shown in Fig. 1. Importantly, two cell lines, ELT 6 and ELT 10, were resistant to growth inhibition by tamoxifen at these same concentrations.

Because responsiveness to tamoxifen varied between the cell lines, the potential role of growth factors as mediators of the action of tamoxifen in these cells was investigated further. Estrogen receptor expression and growth factor expression by the cell lines was examined. As shown in Table 1, a good correlation emerged between responsiveness to tamoxifen and IGF-I expression.
The action of tamoxifen as an estrogen antagonist is believed to be classically responsible for growth inhibition by this drug. The results described above, however, indicate that growth inhibition by tamoxifen did not correlate with estrogen receptor expression by the ELT cell lines (Table 1). Of the cell lines examined, all those inhibited by tamoxifen expressed IGF-I, and both nonresponsive lines did not express this growth factor. In addition to the growth inhibition seen in ELT 3, ELT 4, and ELT 9, two cell lines derived from spontaneous leiomyosarcomas (27) were also found to express IGF-I and its receptor, and these cell lines were also growth inhibited by tamoxifen in vitro (data not shown). No such correlation was seen for any of the other mediators of leiomyoma growth examined (Table 1), indicating that the growth-inhibitory effects of tamoxifen were specific for IGF-I expression.

To confirm this correlation, IGF-I-positive and -negative ELT cells were treated with the biologically active metabolite 4-hydroxytamoxifen. The estrogen receptor-positive, IGF-I-positive cell line ELT 3 has been reported previously to be sensitive to concentrations of 4-hydroxytamoxifen ranging from 0.01 to 100 nM (32). Similar experiments were conducted with the estrogen receptor-positive (27), IGF-I-negative cell line ELT 10 and are shown in Fig. 2. After 96 h of continuous exposure to the tamoxifen metabolite, the growth of ELT 9 cell lines was not affected significantly by any of the 4-hydroxytamoxifen concentrations that were utilized.

**IGF-I Autocrine Loop.** Northern analysis of the leiomyoma lines indicated that IGF-I was expressed by some but not all of the cell lines. As shown in Fig. 3, the three cell lines responsive to tamoxifen (ELT 3, 4, and 9) expressed the expected 7.5-, 1.9-, and 1.2-kb transcripts for IGF-I, whereas the two cell lines not growth inhibited by tamoxifen (ELT 6 and 10) did not express this growth factor. All of the lines expressed IGF-I receptor transcripts, with predominant transcripts of 10.5 and 7.0 kb (Fig. 3).

The presence of transcripts for both IGF-I and its cognate receptor in the same cell lines raised the possibility that an IGF-I autocrine loop existed in the leiomyoma cells. To determine whether leiomyoma cells produce and secrete IGF-I protein, an IGF-I RIA was performed on conditioned medium harvested from ELT 3 cells grown in the absence of serum. This cell line was found to secrete IGF-I into the basal medium (Fig. 4). The greatest increase in secretion was seen between 72 and 96 h of growth in basal medium, correlating to the period when cells were in log growth. At 96 h, 0.57 ng/ml of IGF-I was detected in the medium, which compares favorably with other cell lines that are known to secrete IGF-I (45–47).

To determine whether ELT 3 cells were secreting biologically active IGF-I, serum-free conditioned medium from these cells was tested for the ability to replace IGF-I in stimulating the growth of human mammary epithelial cells (MCF-10A cells). MCF-10A cells have an absolute requirement for IGFs for proliferation in serum-free medium, respond to IGF-I in the sub-nanogram per milliliter range, and respond maximally to IGF-I in the range of 10–25 ng/ml (43, 44). MCF-10A cells were grown in serum-free medium devoid of IGF-I or in this same medium supplemented with ELT 3-conditioned medium at 50% v/v. MCF-10A cells were quiescent in medium devoid of IGF-I but underwent a greater than 2-fold increase in cell number over the 7 days of the experiment when cultured in 50% ELT 3-conditioned medium (Fig. 5).

The presence of functional IGF-I receptor was confirmed in cell line ELT 3 by demonstrating that exogenous IGF-I was mitogenic for these cells. Utilizing medium devoid of insulin and containing 0.5% serum, IGF-I at concentrations of 10–100 ng/ml produced a dose-dependent increase in cell number that was significant at all concentrations tested (Fig. 6).

To establish a role for endogenous IGF-I as an autocrine growth factor for leiomyoma cells, neutralizing antibody directed against IGF-I was added to serum-free cultures of ELT 3 cells. As shown in Fig. 7, antibody to IGF-I at a concentration of 30 ng/ml resulted in a 30% decrease in the growth of these cells after 72 h of continuous antibody exposure, indicating a dependence of the cells on endogenous IGF-I production for maximum cell proliferation.

**DISCUSSION**

These experiments describe the ability of tamoxifen to modulate the growth of leiomyoma-derived cell lines. In five of seven lines examined, including two cell lines derived from leiomyosarcomas, a similar pattern of growth inhibition by tamoxifen was observed. A correlation was found between growth inhibition by tamoxifen and IGF-I expression by the cell lines. Each of the leiomyoma lines and the two

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**Table 1** Correlation between tamoxifen responsiveness and IGF-I expression in the ELT cell lines

<table>
<thead>
<tr>
<th>ELT 3</th>
<th>ELT 4</th>
<th>ELT 6</th>
<th>ELT 9</th>
<th>ELT 10</th>
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<tbody>
<tr>
<td>% inhibition by tamoxifen*</td>
<td>41.3 ± 8.8%</td>
<td>16.3 ± 2.6%</td>
<td>No inhibition</td>
<td>19.0 ± 8.0%</td>
</tr>
<tr>
<td>2.5 μM</td>
<td>59.3 ± 11.0%</td>
<td>27.7 ± 11.5%</td>
<td>No inhibition</td>
<td>32.0 ± 4.9%</td>
</tr>
<tr>
<td>5.0 μM</td>
<td>+</td>
<td>+</td>
<td>No expression</td>
<td>+</td>
</tr>
<tr>
<td>IGF-I expression</td>
<td>+</td>
<td>+</td>
<td>No expression</td>
<td>+</td>
</tr>
<tr>
<td>Estrogen receptor (27)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>IGF-I receptor</td>
<td>No expression</td>
<td>No expression</td>
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<tr>
<td>IGF-II expression</td>
<td>No expression</td>
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<td>PDGF A expression</td>
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<td>PDGF B expression</td>
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<td>TGFα expression</td>
<td>+</td>
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<td>EGF expression</td>
<td>+</td>
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* The percentage of growth inhibition by tamoxifen is shown as the mean ± SEM for three independent experiments.
growth factor to act as a mitogen in the cell type in question, and (c) demonstration that interruption of the autocrine loop, typically with a neutralizing antibody to the growth factor or its receptor or with antisense oligonucleotides, inhibits cell proliferation. In this regard, we demonstrated that (a) transcripts for IGF-I and its receptor were expressed in the cell lines, and IGF-I was produced by the leiomyoma cells and secreted into the culture media; (b) IGF-I was mitogenic for the leiomyoma cells; and (c) neutralizing antibody to IGF-I could inhibit cell proliferation. In the ELT 3 cell line, transcripts for both IGF-I and the IGF-I receptor are detectable, consistent with the hypothesis that an IGF-I autocrine loop exists in these cells. In addition, a RIA for the growth factor detected 0.57 ng/ml of IGF-I in conditioned medium harvested from ELT 3 cells grown under serum-free conditions.

The bioassay for IGF-like activity in MCF-10A cells is very specific for IGFs, including insulin, IGF-I, and IGF-II. Other growth factors such as EGF-like factors, PDGFs, and fibroblast growth factors cannot substitute for the IGF family of growth factors. ELT 3 cells do not express transcripts for IGF-II, suggesting that the stimulation of proliferation in MCF-10A cells with ELT 3-conditioned medium is due to IGF-I in that medium. The bioassay data are consistent with the RIA data and demonstrate that the IGF-I measured

Establishment of an autocrine loop typically requires the satisfaction of several criteria, including: (a) expression of the growth factor and its cognate receptor in the transformed cells; (b) the ability of the

leiomyosarcoma cell lines that were growth inhibited by tamoxifen expressed IGF-I, whereas the two nonresponding cell lines did not. Responsiveness to tamoxifen did not correlate with estrogen receptor status in those same cell lines: one cell line (ELT 10), for example, expressed estrogen receptor but not IGF-I and was not growth inhibited by the antiestrogen or its more potent metabolite. This observation suggests that tamoxifen may act by interrupting an IGF-I autocrine loop in the cell lines.

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IGF-I AND TAMOXIFEN RESPONSE IN LEIOMYOMA

Inhibits the mitogenic effects of IGF-I in the rat leiomyoma cells by inhibiting IGF-I bioavailability.

The ability of neutralizing antibody to IGF-I to inhibit ELI 3 proliferation under serum-free conditions demonstrates that the level of secreted IGF-I is physiologically significant and capable of stimulating growth in the leiomyoma cells. This growth inhibition by the antibody is specific, given that the IGF-I-negative cell line ELI 6 is not growth inhibited by antibody directed against IGF-I under identical experimental conditions (data not shown). Taken together, these data establish the existence of an IGF-I autocrine loop in leiomyoma cells.

Although a paracrine role has been suggested for IGF-I in mediating stromal-myometrial interaction in the normal myometrium (14),

Fig. 4. Production of IGF-I by RIA. ELI 3 cells at 50% confluence were placed in basal medium without serum or insulin. The medium was then collected every 24 h (□), concentrated, and analyzed by RIA (see text for details).

Fig. 5. IGF-I-like mitogenic activity in ELI 3-conditioned media. IGF-I-dependent MCF-10A cells were cultured in serum-free medium supplemented with EGF and hydrocortisone. Negative control cells were grown in the absence of IGF-I (SFHE); test cells were grown in serum-free medium without IGF-I and with ELI 3-conditioned medium at 50% v/v. The cells were grown for 7 days with two changes of medium, and the number of cells per well was determined by counting isolated nuclei with a Coulter counter. Columns, mean number of cells per well for triplicate wells; bars, S.E. *, significantly different from control values at P < 0.05.

by RIA is biologically active and capable of stimulating growth in IGF-requiring cell lines.

IGF-I receptor transcripts were demonstrated in the cell lines, and the presence of functional receptor was verified by the ability of exogenous IGF-I to stimulate proliferation in the ELI 3 cell line. The approximately 20% increase in proliferation seen after 72 h of exposure to 20 ng/ml of IGF-I is somewhat less than the 32% increase seen in MCF-7 human breast cancer cells after the same time period (48). Differences in media and serum composition between investigators could partially explain the differences in responsiveness between cell types. In addition, rat cells have been reported to express high levels of IGFBPs, and it may also be that expression of these proteins inhibits the mitogenic effects of IGF-I in the rat leiomyoma cells by inhibiting IGF-I bioavailability.

Fig. 6. Mitogenic effects of IGF-I in ELI 3 cells. Growth kinetics were determined by counting cell numbers treated with varying concentrations of human recombinant IGF-I. Cells were grown in 10–100 ng/ml IGF-I in medium devoid of insulin and containing 0.5% serum. Data shown were obtained after 72 h of exposure to IGF-I and are representative of three independent experiments. *, significantly different from control values at P < 0.05; bars, S.E.

Fig. 7. Neutralizing antibody to IGF-I. Growth kinetics were determined by counting cell numbers in serum-free medium containing antihuman IGF-I or an isotype-matched IgG (control Ab). Data shown are representative of three independent experiments; bars, S.E.
our data are the first to demonstrate an autocrine role for IGF-I in leiomyoma cells. The potential for IGF-I to act as an autocrine growth factor for these cells has been suggested previously for human uterine leiomyomas (49). Human myometrium expresses IGF-I, and transcripts for the growth factor are elevated significantly in leiomyomas versus adjacent normal myometrium (50–52). IGF-I receptor is also present at elevated levels in leiomyomas (53), suggesting that the IGF-I signaling pathway is altered in these tumors. In addition, although no extensive information is available on IGFBP expression in normal myometrium, preliminary data by others indicate that the pattern of binding protein expression may differ between normal myometrium and uterine leiomyoma (49). These data are also consistent with the hypothesis that alterations in an IGF-I signaling pathway may contribute to myometrial tumor development.

In light of its potential role in leiomyoma growth, IGF-I is of particular interest as a mediator of tamoxifen effects. IGF-I is mitogenic for many breast cancer cells, and tamoxifen-responsive, estrogen receptor-negative tumor types have been shown to express receptors for IGF-I (20). Tamoxifen has been shown to reduce levels of serum IGF-I when administered p.o. to women (24, 54–57). In addition, when administered in an in vivo experimental system used previously to demonstrate its cytostatic effect on breast cancer cell proliferation, tamoxifen was shown to inhibit the expression of the IGF-I gene locally in liver and lung (20). This inhibition was partly pituitary independent, suggesting that tamoxifen may be acting to reduce IGF-I expression locally in these organs. Although a recent manuscript describes an increase in rat uterine IGF-I after tamoxifen administration (58), that work examined total uterine RNA rather than separating the uterus into endometrial and myometrial components. Tamoxifen has been associated with an increased risk of endometrial cancer and may act as an estrogen agonist in this tissue type (59). Previous work (32) and the present studies revealed no such agonist activity in the myometrium, thus making an important distinction between endometrial and myometrial tamoxifen effects.

In addition to its effects on local IGF-I production, tamoxifen has been shown to modulate IGFBP expression at the RNA level in MCF-7 breast carcinoma cells in tissue culture (60). Tamoxifen administration is also associated with modulation of IGFBP levels in conditioned medium using Western ligand and immunoblot analysis (61). In those experiments, tamoxifen increased levels of IGFBP3 versus controls. In addition, tamoxifen has been shown to increase circulating levels of IGFBP1 in women treated with this drug (56). The ability of tamoxifen to modulate IGFBP levels in other systems suggests that the effects of this compound in uterine leiomyomas could also be mediated in part through modulation of IGFBP expression.

Clinical trial data indicate that the efficacy of tamoxifen in treating breast cancer does not depend fully on the presence of estrogen receptor in the tumor in question (62). The compound has some therapeutic benefit in a certain percentage of estrogen receptor-negative breast tumors and may therefore operate via a mechanism of action independent of its ability to act as an estrogen antagonist (25). This is the first report of the ability of tamoxifen to inhibit proliferation in transformed myometrial cells and demonstrates that this activity may also be independent of estrogen receptor status in those cells. Tamoxifen, therefore, may act mechanistically in fibroids in a fashion not solely dependent on the ability of the cell to see tamoxifen as an estrogen antagonist perhaps by interrupting an IGF-I autocrine loop in these cells. Whether tamoxifen is acting to suppress leiomyoma cell growth at the level of IGF-I expression or the level of IGF-I bioavailability is not known at this time. Future experiments utilizing the Eker rat model will be able to further elucidate the mechanism by which tamoxifen inhibits cell growth of this clinically important tumor type.

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