Role of Hormonal and Reproductive Factors in the Etiology and Treatment of Uterine Leiomyoma

CHERYL L. WALKER

The University of Texas M.D. Anderson Cancer Center, Department of Carcinogenesis, Science Park-Research Division, Smithville, Texas 78957

ABSTRACT

Uterine leiomyomas are the most common gynecologic neoplasm in reproductive-age women. While it is clear that hormonal factors play a prominent role in this disease, how steroid hormones contribute to disease etiology or may be utilized as targets for intervention are currently areas of active scientific investigation. To study the impact of hormones on uterine leiomyomas, the Eker rat has been developed as an *in vivo*/*in vitro* animal model system for these tumors. Spontaneous leiomyomas arise in intact Eker rats with a high frequency and leiomyoma-derived cell lines from these animals maintain the biochemical and physiological characteristics of the tumors from which they were obtained. Using this animal model system, it has been established that tumor development is absolutely dependent on steroid hormones and that sensitivity/responsiveness to estrogen is enhanced in tumors and tumor-derived cell lines. Modulation of hormonal milieu, such as that which naturally occurs during pregnancy, can effectively inhibit tumor development. The hormone responsiveness of these tumors makes them good candidates for hormonal therapy. Selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene hold promise as potential therapeutic agents for this disease. SERMs inhibit proliferation of leiomyoma-derived cell lines *in vitro*, repress the growth of these lines in nude mice, and, when administered over a 2- to 4-month course of treatment to Eker rats, reduce tumor incidence by more than 50%. In addition to endogenous hormones, xenoestrogens in our environment (e.g., phytoestrogens, organochlorine pesticides, pharmacologic compounds) are of potential concern with regards to their impact on this disease. These environmental estrogens have been shown to promote the growth of leiomyoma cells *in vitro* and *in vivo*. Further elucidation of the role of these and other hormonal and reproductive factors in the development of uterine leiomyoma will be invaluable for increasing our understanding of the etiology of this disease and developing new therapeutic strategies to help to reduce the negative impact of uterine leiomyomas on women's health.

I. Introduction

Uterine leiomyomas, commonly referred to as "fibroids," are benign tumors arising from the myometrial compartment of the uterus. They are typically well differentiated, have a relatively low mitotic index, and retain their smooth muscle phenotype. Uterine leiomyomas are the most common gynecologic neoplasm, occurring with a remarkable frequency in more than 70% of reproductive age

women (Cramer and Patel, 1990). Tumors can become quite large and are usually multiple. When symptomatic, they are associated with infertility, menorrhagia, and spontaneous abortion and are the leading indication for hysterectomy in premenopausal women (Buttram and Reiter, 1981).

II. The Eker Rat Model for Uterine Leiomyoma

The Eker rat has been extensively characterized as an *in vitro*/*in vivo* animal model for uterine leiomyoma (Everitt *et al.*, 1995; Howe *et al.*, 1995a). Female Eker rats carrying a germline mutation in the tuberous sclerosis 2 (Tsc-2) tumor suppressor gene develop uterine leiomyomas by 12–16 months of age with a frequency of $\approx$ 65% (Everitt *et al.*, 1995). The predisposing genetic alteration in these animals is an insertion of an endogenous retrovirus between exons 30 and 31 of the Tsc-2 gene, which inactivates the tumor suppressor protein encoded by this gene (Yeung *et al.*, 1994; Kobayashi *et al.*, 1995). The uterus is a site of high expression of tuberin, the product of the Tsc-2 gene, and tumors that arise in heterozygous animals show loss of tuberin function. At the DNA level, loss of heterozygosity (LOH) at the Tsc-2 locus is commonly observed in these tumors, with loss of tuberin function occurring by several mechanisms, including loss of chromosome 10 on which the wild-type Tsc-2 gene is located (either monosomy or chromosome nondisjunction with retention of two copies of chromosome 10 containing the mutant allele), gene silencing, and point mutations (Yeung *et al.*, 1995).

Uterine leiomyomas that arise in the Eker rat share many phenotypic characteristics with their cognate human disease. Tumors occur spontaneously with a high frequency in intact cycling females. They are often multiple and resemble human leiomyomas histologically (Everitt *et al.*, 1995). Eker rat leiomyomas are benign and their malignant counterparts, uterine leiomyosarcomas, are rarely seen. The similarity in pathogenesis of uterine leiomyoma in Eker rats and women has made these animals useful as a model system to experimentally address questions related to the role of hormones in tumor development and hormonal and reproductive factors that can modulate or prevent the development of this disease.

III. Eker Leiomyoma Tumor-derived Cell Lines

Several cell lines have been developed from Eker rat uterine leiomyomas (Howe *et al.*, 1995a). These cell lines have been given the designation ELT (for Eker leiomyoma tumor-derived) and, to date, five such lines have been established and characterized (Table I). All five cell lines are positive for expression of smooth muscle α and γ actins and desmin by northern analysis and immunocytochemistry. As shown in Table I, the cell lines vary with respect to steroid

TABLE I
Characteristics of ELT Cell Lines

	ELT-3	ELT-4	ELT-6	ELT-9	ELT-10
Smooth muscle actin	Positive	Positive	Positive	Positive	Positive
Desmin	Positive	Positive	Positive	Positive	Positive
Estrogen receptor	Positive (Kd = 0.7 nM)	Positive	Negative	Positive	Positive
Progesterone receptor	Positive (Kd = 1.3 nM)	Positive	Negative	Positive	Positive
Tumorigenicity in nude mice	Tumorigenic	Nontumorigenic	Nontumorigenic	Nontumorigenic	Nontumorigenic

hormone receptor expression and tumorigenicity, although, in general, these lines continue to express both estrogen and progesterone receptors in culture and are nontumorigenic in nude mice.

IV. Hormone-responsive Phenotype of Uterine Leiomyoma

A substantial body of evidence from human clinical and epidemiological observations and experimental data obtained using the Eker rat model point to the hormone responsiveness of uterine leiomyomas. These tumors are most symptomatic in pre- and perimenopausal women and often change dramatically in size during pregnancy (Rossi and Diamond, 1992; Strobelt *et al.*, 1994). The indication for hysterectomy due to symptomatic fibroids reaches a maximum incidence at 45 years of age, then declines dramatically, coinciding with the onset of menopause (Cramer, 1992). Uterine leiomyomas express estrogen receptors (ERs) and progesterone receptors (PRs) at levels that have been reported to be the same or higher than normal myometrium (Rein and Nowak, 1992; Brandon *et al.*, 1993,1995; Viville *et al.*, 1997; Englund *et al.*, 1998; Nisolle *et al.*, 1999). Treatment with gonadotropin-releasing hormone (GnRH) agonists is a commonly used adjuvant therapy for these tumors. The hypoestrogenic state induced by these compounds effectively shrinks tumor volume (Andreyko *et al.*, 1987; Adamson, 1992). However, the inhibition of steroid hormone production by GnRH agonists can cause a significant loss of bone mineral density (Dawood *et al.*, 1989), limiting the course of treatment to less than 6 months. Moreover, cessation of therapy results in regrowth of these tumors, often rapidly (Friedman *et al.*, 1990).

In the Eker rat model, the dependence of these tumors on ovarian hormones has been demonstrated unequivocally. Ovariectomy at 4 months of age virtually

ablates tumor development, whereas animals receiving sham surgeries have a tumor incidence at 16 months of ≈ 65% (Walker *et al.*, 2000). Similarly, analysis of cell proliferation in normal myometrium and leiomyomas of Eker rats suggests that these tumors have an enhanced responsiveness to the mitogenic effects of estrogens (Burroughs *et al.*, 2000). As shown in Figure 1, in young animals, myometrial cell proliferation correlates well with estrogen levels, being highest when estrogen levels peak during proestrus. As the animal matures, however, myometrial cells become refractory to the mitogenic stimulus of estrogen. This phenomenon appears to be specific for mesenchymal cells of the uterus. While both smooth muscle myometrial cells and endometrial stomal cells become refractory to the proliferative effects of estrogen with age, this is not observed for either luminal or glandular epithelial cells of the endometrium (Burroughs *et al.*, 2000). In contrast, leiomyomas exhibit a significantly elevated proliferative index relative to age-matched normal myometrium, suggesting an enhanced sensitivity to the mitogenic effects of steroid hormones in these tumors (Burroughs *et al.*, 2000). This conclusion is supported by data using human leiomyoma explants, which demonstrated that tumor cells displayed an enhanced sensitivity to estro-

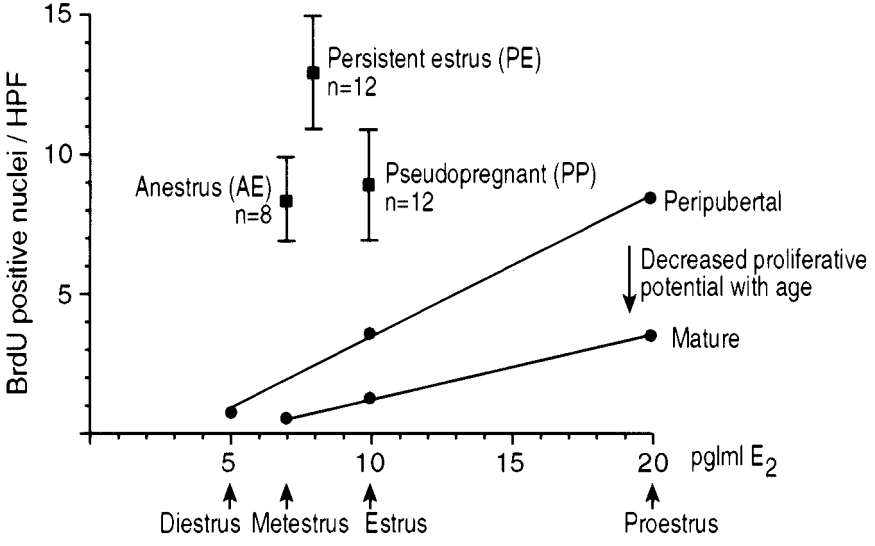

FIG. 1. Cell proliferation in normal myometrium and leiomyomas. Cell proliferation was measured by quantitation of BrdU incorporation into DNA followed by identification of BrdU-positive nuclei by immunohistochemistry using antibodies directed against BrdU. Cell proliferation positively correlated with serum estrogen levels but an overall decrease in the mitogenic response to estrogen occurred in the myometrium with age. Tumors exhibited an increased sensitivity/responsiveness to estrogen relative to normal myometrium.

gen at the transcriptional level, relative to normal myometrial cells (Andersen *et al.*, 1995).

It is important to appreciate that there are species-specific differences in how levels of estrogen and progesterone change during the menstrual cycle of women and the estrus cycle of rodents. In women, estrogen levels exhibit two peaks: one during late-follicular/proliferative phase of the cycle and a second, broader surge during the luteal/secretory phase. In contrast, progesterone levels are elevated only during the luteal phase of the cycle (Figure 2). Thus, while the luteal phase of the menstrual cycle is often considered to be "progesterone driven," it is important to appreciate that total exposure to estrogen during this phase of the cycle is higher or equal to that of the follicular phase in cycling women. In rodents, circulating estrogen levels peak in proestrus and drop precipitously during estrus following ovulation. Although estrogen levels begin to rise again during diestrus, these levels remain very much attenuated relative to the surge in estrogen that occurs during proestrus. In contrast, progesterone levels show a dramatic bimodal pattern. Peaks in progesterone levels occur during both early proestrus and metestrus (Figure 2), so that significant exposure to progesterone occurs in both early and late phases of the cycle. Therefore, in women, highest coincident estrogen and progesterone exposure occurs during the luteal phase of the cycle, whereas, in rodents, the highest coincident estrogen and progesterone levels occur in proestrus.

The biology of leiomyomas in rats and humans reflects this difference in physiology. For example, in leiomyomas, both cell proliferation and apoptosis vary as a function of the menstrual/estrus cycle. In humans, although the data are limited, leiomyomas appear to be most proliferative during the luteal phase of the cycle (Kawaguchi *et al.*, 1989). Subsequent reports tend to support this earlier data (Nisolle *et al.*, 1999; Wu and Somlo, 2000), although in one of these studies, the difference between proliferation occurring in the two phases did not reach statistical significance (Nisolle *et al.*, 1999). In the Eker rat, empirical data have recently been obtained that demonstrate that cell proliferation in these tumors is maximal during proestrus, where hormone levels correspond to the high estrogen and progesterone levels that occur during the luteal phase in women. Conversely, in the normal myometrium, apoptosis is maximal during estrus, when steroid hormones are approaching their nadir. In addition, leiomyomas in the Eker rat model have been demonstrated to have a defective apoptotic program, relative to age-matched normal myometrium (Burroughs *et al.*, 2000). Human data on apoptotic rates in leiomyoma are extremely limited, although there is some suggestion of such a defect from clinical data as well (Huang *et al.*, 1997; Matsuo *et al.*, 1997).

Work with ELT cell lines *in vitro* further supports the contention that leiomyomas have an enhanced sensitivity/responsiveness to steroid hormones. These cells retain estrogen and progesterone receptors and proliferate in response

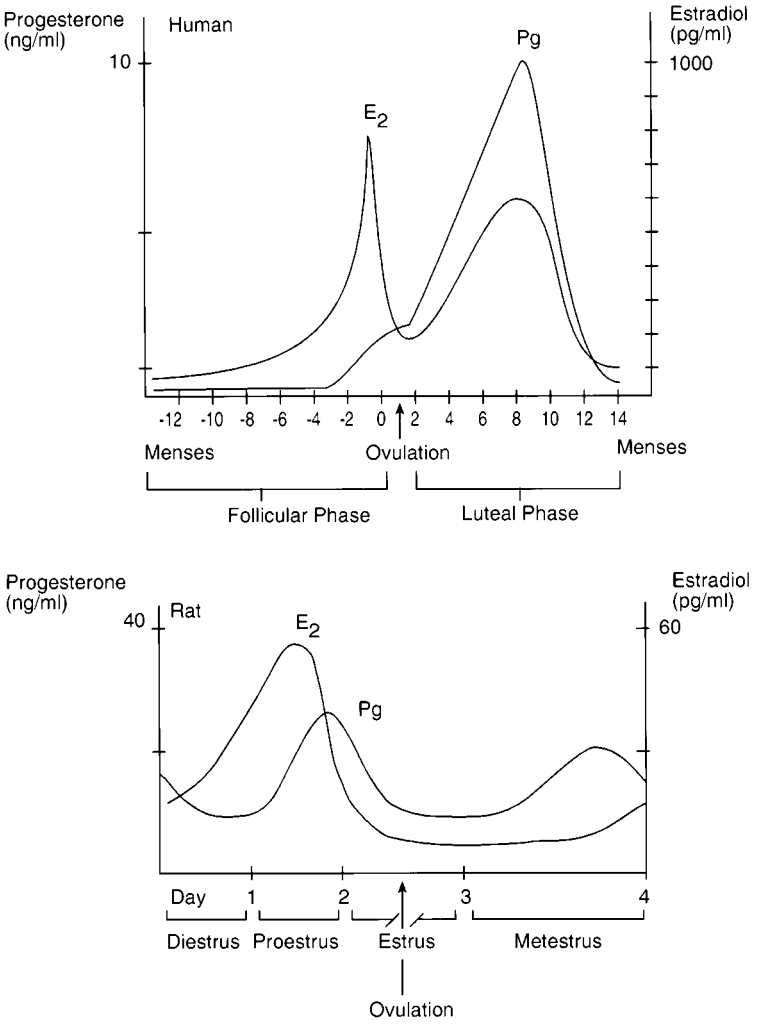

FIG. 2. Comparison of cycling hormone levels during the menstrual and estrus cycles. Species-specific differences occur in the cyclical levels of steroid hormones throughout the menstrual (human) and estrus (rodent) cycles. Estrogen exhibits a bimodal pattern during the menstrual cycle, whereas only a single peak in the level of this hormone occurs during the estrus cycle in rats. Progesterone levels also show differences between the two species, exhibiting a single peak during the menstrual cycle but exhibiting a bimodal pattern during the rat estrus cycle. [Data adapted from Speroff L, Vande Wiele RL 1971 Regulation of the human menstrual cycle. Am J Obstet Gynecol 109:234–247; and adapted with permission from Smith M, Freeman M, Neill J 1975 The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat:prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 96:219–226. Copyright The Endocrine Society.]

to 10^{-6} to 10^{-10} M 17-β-estradiol (Howe *et al.*, 1995b). This is fortuitous and contrasts with human myometrial and leiomyoma cells, which rapidly lose their receptors in tissue culture (Severino *et al.*, 1996). In addition to responding to estrogen *in vitro*, leiomyoma cells also respond to this hormone *in vivo*. When ELT cells are injected subcutaneously into nude mice, they form tumors and grow as xenografts. With estrogen supplementation, mean tumor volume of these xenografts is increased two-fold (Howe *et al.*, 1995b), again demonstrating the estrogen responsiveness of these tumor cells. Although hormone responsive, ELT cells do not require estrogen for cell growth, probably due to the insulin-like growth factor 1 (IGF-I) autocrine loop present in these cells (Howe *et al.*, 1996). However, when deprived of growth factors via serum starvation, ELT cells will growth arrest and undergo apoptosis. Treatment of serum-starved cells with estrogen increases cell number. This rescue is mediated by enhanced cell proliferation rather than inhibition of apoptosis, which occurs at the same rate in the presence and absence of estrogen (Burroughs *et al.*, 1997). Thus, estrogen appears to be a mitogen for leiomyomas that can modulate cell proliferation but has little effect on the apoptotic program of these tumors.

V. Hormonal and Reproductive Factors That Modulate Tumor Development

Several endogenous or environmental factors that modulate risk for developing uterine leiomyoma affirm the hormone responsive nature of this disease. Obesity and age at menarche have been linked to an increased risk for uterine leiomyoma, while cigarette smoking, use of oral contraceptives containing progesterone, and parity have been identified as protective factors (Ross *et al.*, 1986; Parazzini *et al.*, 1988,1992,1996a,b; Kjerulff *et al.*, 1996, Marshall *et al.*, 1997,1998a,b; Chiaffarino *et al.*, 1999a,b). Obesity, particularly in peri- and postmenopausal women, increases levels of circulating estrogen through aromatization of fat stores, while early menarche increases overall exposure to circulating ovarian hormones. Cigarette smoking induces enzymes that can promote estrogen metabolism. The progesterone present in estrogen + progesterone oral contraceptives opposes the action of estrogen present in these formulations. In the case of pregnancy, the risk of uterine leiomyoma in parous women is approximately half that of nulliparous women. The risk of developing this disease decreases significantly with increased number of pregnancies (Ross *et al.*, 1986; Parazzini *et al.*, 1988,1996a,b). However, epidemiological data have been subject to interpretation as to whether pregnancy *per se* is protective or, as leiomyomas are a major cause of infertility, women that develop these tumors are less fertile and thus have lower pregnancy rates. Although the mechanism by which parity acts as a protective factor is not yet clear, additional experimental

data obtained in the Eker rat model suggest that the hormonal milieu associated with pregnancy may help prevent tumor development (Table II).

Nulliparous, virgin female Eker rats develop leiomyomas with a frequency of 65%. When these animals are bred with fertile males, a dramatic shift in tumor incidence and presentation is observed. As shown in Table II, in females allowed to have a single litter to confirm fertility, tumor incidence was 71% for gross and microscopic lesions combined, whereas in animals undergoing multiple rounds of pregnancy (average five litters/animal), tumor incidence was reduced to $\approx$ 10%. This protective effect was particularly pronounced for tumors of the uterine cervix, the primary site of tumor development in fertile/nulliparous animals ($\approx$ 80%), whereas less than 15% of the tumors arose in this location in animals undergoing multiple pregnancies (Walker *et al.*, in press) (Figure 3).

Pregnancy has been shown to modulate the incidence of other types of hormone-dependent tumors, although the mechanisms of this protective effect appear to operate at different levels in different cell types. In breast cancer, early pregnancy is the most protective, suggesting an effect on the normal target cell population from which these tumors arise (Colditz, 1993; Hulka, 1996; Kelsey and Bernstein, 1996; Adami *et al.*, 1998). Experimental animal studies have confirmed this hypothesis and suggest that differentiation of ductal epithelial

TABLE II
Impact of Hormonal and Reproductive Factors on Uterine Leiomyoma

Condition	Ovariectomy	SERM treatment (antiestrogen)	Targertin treatment (RXR ligand, antiestrogen)	Pregnancy
Expected tumor incidence	Gross + micro 23/34 = 68% (sham surgery)	Gross + micro 26/31 = 84% (placebo) 23/37 = 62% (vehicle)	Gross = 8/24 Micro = 1/24 (vehicle)	Gross + micro 12/17 = 71% (single-litter fertile females)
Observed tumor incidence	Gross + micro 1/31 = 3%	Gross + micro 10/29 = 35% (tamoxifen) 11/30 = 37% (LY 326315)	Gross = 4/38 Micro = 9/38	Gross + micro (in parous rats having 4–5 litters) 6/58 = 10%
Modulation	95% decrease in tumor incidence ($p \leq 0.001$)	40–60% decrease in tumor incidence ($p \leq 0.05$)	65% decrease in macroscopic lesions ($p \leq 0.05$)	86% decrease in tumor incidence ($p \leq 0.001$)
Reference	Walker *et al.*, 2000	Walker *et al.*, 2000	Gamage *et al.*, 2000	Walker *et al.*, in press

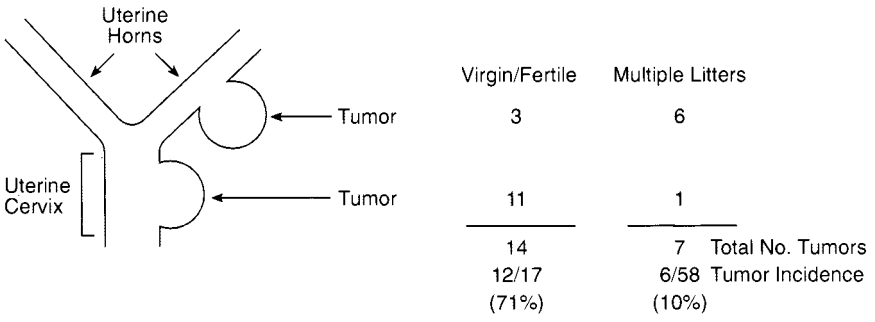

FIG. 3. Presentation of leiomyomas as a function of parity. In addition to reducing tumor incidence, pregnancy changes the spatial distribution of tumors. Virgin animals and those having a single litter (to confirm fertility) primarily develop tumors within the uterine cervix, whereas those undergoing multiple pregnancies develop tumors primarily on the uterine horns.

cells in the mammary gland mediated by the hormones of pregnancy contributes significantly to the protective effect of this condition (Abrams *et al.*, 1998; Russo and Russo, 1998; Sivaraman *et al.*, 1998; Guzman *et al.*, 1999). In contrast, in the endometrium, time of last pregnancy appears to be a more important determinant. In women with only a single pregnancy, the most protection is afforded to women having a late rather than early pregnancy. This suggests that the protective effect is acting against the nascent neoplastic/preneoplastic cell population (Albrektsen *et al.*, 1995; Hirose *et al.*, 1996; Franchesschi, 1998; Lambe *et al.*, 1999; Parslov *et al.*, 2000). The fact that no protection against the development of uterine leiomyoma was afforded female Eker rats undergoing a single early pregnancy would suggest that the protective mechanisms of pregnancy in the myometrium are more similar to the endometrium (i.e., acting against nascent tumor cells) than the breast (protective differentiation).

Anderson and Barbieri first suggested an interesting hypothesis in which leiomyoma cells were described as resembling a myometrial cell of pregnancy rather than a typical myometrial cell under the influence of the menstrual cycle (Andersen *et al.*, 1995). These similarities include increased levels of steroid hormone receptors, expression of IGF-I, and production of extracellular matrix proteins such as collagens type I and II. Furthermore, leiomyomas constitutively express high levels of connexin 43, the predominant connexin found in the myometrium during pregnancy (Andersen *et al.*, 1993). Connexin 43 expression is negligible in myometrial cells during the normal menstrual cycle and early pregnancy; however, at term, connexin 43 becomes abundantly expressed under the influence of estrogen (Chow and Lye, 1994). This confirms that leiomyomas not only share the characteristics of myometrial cells during pregnancy but more specifically have the phenotype of these cells at parturition. At parturition, myometrial smooth muscle cells have a unique phenotype that differentiates

them from myometrial cells at other stages of pregnancy. High levels of expression of a number of contraction-associated proteins (e.g., connexin 43, oxytocin receptors, cycloxygenase and its associated prostaglandins) are unique to the parturient myometrial cell. Leiomyoma cells share this phenotype but differ from the parturient myometrium in that they are neoplastic. Key biochemical and molecular differences must exist between a myometrial cell at parturition and a leiomyoma cell to account for this difference. An example of one such fundamental difference between myometrial cells and leiomyomas is their ability or inability, respectively, to undergo the biological program responsible for remodeling and involution of the uterus that occurs in a pregnant myometrial cell following parturition. In leiomyoma cells, this deficiency may be due to their hypersensitivity to steroid hormones, which may prevent them from triggering the normal dedifferentiation or apoptotic program of a parturient myometrial cell and returning to a nongravid, quiescent state (Cesen-Cummings *et al.*, 2000).

Selective estrogen receptor modulators (SERMs) are a new class of synthetic compounds that bind to the estrogen receptor and exhibit tissue-specific agonist or antagonist activity (Jordan and Furr, 2001). Compounds such as tamoxifen and raloxifene are two prototypical SERMs currently in clinical use for prevention/treatment of breast cancer and osteoporosis (Nayfield *et al.*, 1991; Jordan, 1992; Fuchs-Young, 2001). Tamoxifen is a nonsteroidal triphenylethylene that is metabolized *in vivo* to several intermediates, the principal one being N-desmethyltamoxifen. Raloxifene is a benzothiophene derivative that binds to the estrogen receptor with nanomolar affinity and, like tamoxifen, acts as an estrogen agonist or antagonist, depending on cellular context. The tissue specificity observed for these compounds has been exploited to provide antagonism of estrogen action in the breast, while avoiding adverse side effects in other hormone-responsive tissues. Unfortunately, in the uterus, tamoxifen acts as an agonist in the endometrium and can promote the development of endometrial carcinoma (Hardell, 1988; Fornander *et al.*, 1989; Kedar *et al.*, 1994; Hulka, 1997). However, until recently, the effect of these or other SERMs on the myometrium and on leiomyomas had not been investigated. Preclinical data obtained from the Eker rat model now suggest that this class of compounds may be effective therapeutic agents for leiomyoma.

In culture, ELT cell lines proliferate in response to estrogen. Both basal and estrogen-induced cell proliferation are inhibited by 4-OH tamoxifen (a biologically active metabolite of tamoxifen) and raloxifene (Howe *et al.*, 1995b,1996; Fuchs-Young *et al.*, 1996). When these cells are grown as xenografts in nude mice, estrogen treatment increases tumor size, whereas tamoxifen treatment decreases tumor size and increases tumor latency (Howe *et al.*, 1995b). Furthermore, *in vivo* studies in which Eker rats were treated with either tamoxifen or raloxifene for 2–4 months demonstrated that SERMs reduced tumor incidence by $\approx$ 50% and significantly reduced the size of the remaining tumors (Walker *et*

al., 2000) (Table II). Importantly, sectioning of uteri and examination of microscopic lesions in treated rats confirmed a reduction in the absolute number of persistent lesions. This contrasts with the clinical experience with GnRH agonists, which primarily reduce tumor size but do not decrease tumor cellularity (Cohen *et al.*, 1994). These preclinical data in the Eker rat suggest that, in contrast to GnRH agonists, SERMs such as tamoxifen and raloxifene, which both protect the bone and cardiovascular system and ablate leiomyomas, may have efficacy as medicinal therapeutic agents for leiomyoma. In support of the potential efficacy of SERMs, raloxifene recently has been reported to shrink leiomyoma volume in postmenopausal women (Palomba *et al.*, 2001).

An interesting aspect of the preclinical studies with SERMs was the finding that while tumors from treated animals were significantly smaller and had a reduced mitotic index, there was no change in the apoptotic indices of the treated tumors relative to vehicle-treated controls (Walker *et al.*, 2000). SERMs acting as anti-estrogens in the myometrium and leiomyomas were able to inhibit cell proliferation but were ineffective at inducing apoptosis. These *in vivo* data are consistent with the description of leiomyoma cells as having an apoptotic defect that is refractory to modulation by estrogen. However, in another preclinical study, the retinoid X receptor (RXR) ligand targretin (LGD 1069) was shown to be effective at inducing apoptosis in these cells (Table II) (Gamage *et al.*, 2000). Targretin previously has been demonstrated to act as an estrogen antagonist and to have efficacy as a chemopreventive agent for hormone-dependent mammary cancer (Gottardis *et al.*, 1996; Fitzgerald *et al.*, 1997). Treatment of tumor-bearing Eker rats for 4 months with targretin decreased the number of grossly observable tumors and significantly increased tumor apoptotic indices (Gamage *et al.*, 2000). These data suggest that, while refractory to the influence of traditional anti-estrogens, the apoptotic program of leiomyomas may be modulated via other signaling cascades, such as that induced via RXRs.

VI. Potential Impact of Environmental Estrogens

Xenoestrogens with endocrine-disrupting activity have been associated with the dysregulation of reproductive function and promotion of malignancies in experimental animals and human populations. With the recognition of the ubiquitous presence of many of these xenoestrogens in our environment, the high incidence of uterine leiomyomas in women has called into question the potential influence of xenoestrogens in the pathogenesis of these tumors. Using ELT cell lines, several *in vitro* assays have been developed to assess the estrogen-like agonist activity of potential endocrine disruptors on leiomyoma cells. These assays demonstrate that compounds from three major classes of xenoestrogens can mimic the effect of estrogen on leiomyoma cells and act as ER agonists: phytoestrogens, organochlorine pesticides, and pharmacologic agents. These

compounds can stimulate proliferation of ELT cells in culture and transactivate expression of an ERE-reporter construct and an endogenous estrogen-responsive gene, the progesterone receptor, in leiomyoma cells (Hunter *et al.*, 1999; Hodges *et al.*, 2000). Diethylstilbestrol, the phytoestrogens coumesterol, genistein, and narigenin, and the organochlorine pesticides endosulfan, kepone, and HPTE (the active metabolite of metoxychlor) are all able to stimulate cell proliferation in estrogen-deficient medium. Additionally, the organochlorine pesticides methoxychlor, dieldrin, and toxaphene, while unable to stimulate cell proliferation, were able to act as agonists at the molecular level to transactivate the ER to induce expression of both ERE reporter genes and transcription of the PR. Interestingly, agonist activity by these compounds in leiomyoma cells requires activation of both AF1 and AF2 functions of the ER. Compounds that activate AF1 but fail to activate AF2, 4-OH tamoxifen, and two raloxifene analogs act as antagonists in these cells (Hunter *et al.*, 1999).

Significantly elevated levels of organochlorine pesticides previously have been detected in tumors and blood samples of women with leiomyoma (Saxena *et al.*, 1987). These studies suggest a possible link between organochlorine pesticide exposure and the development of uterine leiomyomas. Organochlorine pesticides tend to bioaccumulate in fat stores and to be released during lactation and times of stress such as fasting. Under these conditions, exposure levels may be substantially higher within the body than those originally encountered in the environment. Furthermore, windows of susceptibility may exist at times when circulating estrogen levels are low. Exposure to even relatively low levels of xenoestrogens during windows of susceptibility, such as during the neonatal period, could produce deleterious effects on the reproductive system and potentially contribute to the development of leiomyomas. Several studies have implicated exposure to organochlorine pesticides such as dieldrin with an increased incidence of breast cancer (Wolff *et al.*, 1993; Hoyer *et al.*, 1998), although these data are controversial and recently have been called into question (Hunter *et al.*, 1999).

VII. Summary

It is clear that hormonal milieu has a tremendous impact on the growth and development of uterine leiomyoma. Using experimental animal models such as the Eker rat, it is possible to dissect these hormonal factors and determine their influence on specific aspects of tumor etiology and treatment in ways that are difficult or impossible to approach in humans. As shown in Figure 4, using the Eker rat model, it has been possible to clearly demonstrate that the development of leiomyoma is dependent on steroid hormones and this process can be modulated by several hormonal and reproductive factors. Tumor development is promoted by steroid hormones, and possibly by xenoestrogens, while other

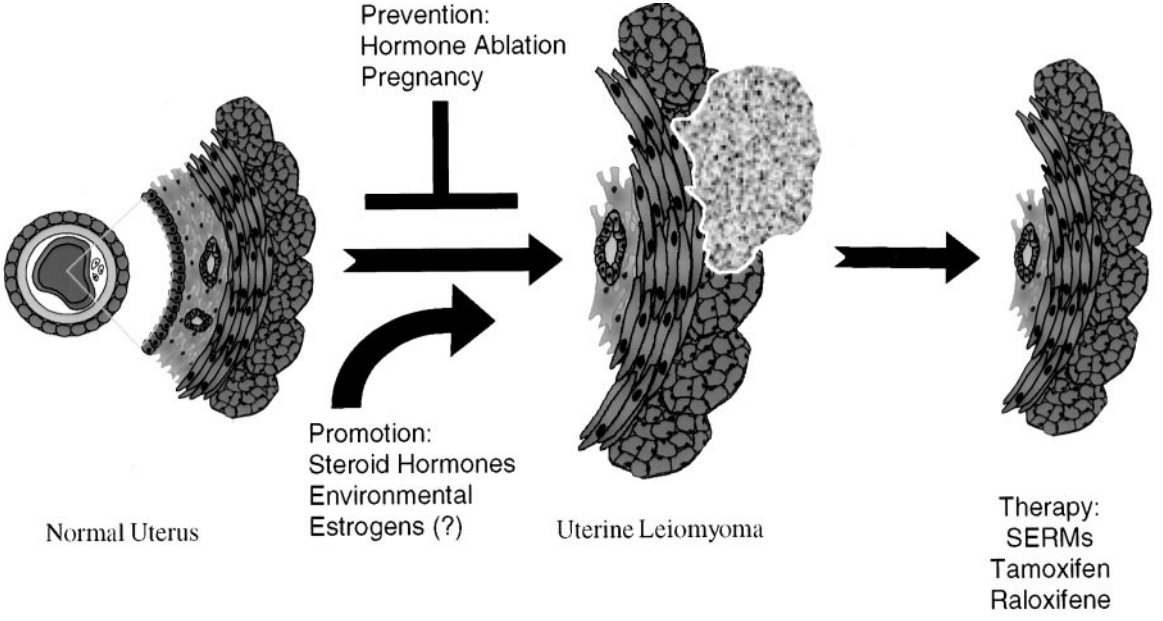

FIG. 4. Hormonal and reproductive factors that impact uterine leiomyomas. Uterine leiomyomas are hormone-dependent tumors and the development of this disease can be promoted by estrogens and inhibited by hormone ablation and pregnancy. SERMs have been demonstrated in preclinical studies to be effective at inhibiting tumor growth and offer promise as new therapeutic agents for this disease.

alterations in a women's hormonal milieu, such as that which occurs during pregnancy, may prevent the development of these tumors. Because leiomyomas retain their hormone-responsive phenotype, they are amenable to hormonal therapy. Traditional adjuvant therapy with GnRH agonists has been demonstrated to reduce tumor burden and symptoms but induces serious side effects associated with the generalized hypoestrogenic milieu these drugs induce. Preclinical studies in the Eker rat model indicate that SERMs, which act as estrogen antagonists in leiomyomas while acting as estrogen agonists in the bone and cardiovascular system, hold promise as new candidate therapeutic agents for this disease.

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