Etiology and Pathogenesis of Uterine Leiomyomas: A Review

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Uterine leiomyomas, or fibroids, represent a major public health problem. It is believed that these tumors develop in the majority of American women and become symptomatic in one-third of these women. They are the most frequent indication for hysterectomy in the United States. Although the initiator or initiators of fibroids are unknown, several predisposing factors have been identified, including age (late reproductive years), African-American ethnicity, nulliparity, and obesity. Nonrandom cytogenetic abnormalities have been found in about 40% of tumors examined. Estrogen and progesterone are recognized as promoters of tumor growth, and the potential role of environmental estrogens has only recently been explored. Growth factors with mitogenic activity, such as transforming growth factor-β_3, basic fibroblast growth factor, epidermal growth factor, and insulin-like growth factor-I, are elevated in fibroids and may be the effectors of estrogen and progesterone promotion. These data offer clues to the etiology and pathogenesis of this common condition, which we have analyzed and summarized in this review. *Key words:* estrogen, fibroids, genetics, growth factors, progesterone, risk factors. *Environ Health Perspect* 111:1037–1054 (2003). doi:10.1289/ehp.5787 available via *http://dx.doi.org/* [Online 13 November 2002]

Uterine leiomyomas, or fibroids, are the most common tumors of women in the United States, probably occurring in the majority of women by the time they reach menopause and becoming clinically significant in about one-third of these women. Despite their prevalence, little attention has been directed toward the causation and pathogenesis of fibroids until recent years because of the rarity of their malignant transformation. Regardless of their generally benign neoplastic character, uterine fibroids are responsible for significant morbidity in a large segment of the female population. The clinical effects of these tumors are related to their local mass effect, resulting in pressure upon adjacent organs, excessive uterine bleeding, or problems related to pregnancy, including infertility and repetitive pregnancy loss (Haney 2000). As a consequence of these local pressure effects and bleeding, uterine fibroids rank as the major reason for hysterectomy in the United States, accounting for approximately one-third of all hysterectomies (Wilcox et al. 1994), or about 200,000 hysterectomies per year (Gambone et al. 1990).

Although the cause or causes of fibroids are unknown, the scientific literature now contains a sizeable body of information pertaining to the epidemiology, genetics, hormonal aspects, and molecular biology of these tumors. In this review we have analyzed and summarized the data available, with the goal of achieving a better understanding of the factors related to the etiology and pathogenesis of fibroids.

In considering the development of uterine leiomyomas, it is convenient to subdivide the factors that may be related to tumorigenesis into four categories: predisposing or risk factors, initiators, promoters, and effectors. Risk factors are characteristics associated with a condition, generally identified by epidemiologic studies. Knowledge of such predisposing factors may provide clues to the etiology of these tumors as well as to preventive measures. The initiators of fibroids are unknown; however, a few of the theories of initiation offered in the literature are briefly reviewed in this article. The occurrence of genetic aberrations in fibroid tumors is considered. Despite the abundance of cytogenetic investigations, uncertainty remains as to the primary or secondary nature of these genetic changes and their impact on the initiation and/or promotion of these tumors. The role of growth promoters of fibroids seems to belong in large part to the ovarian hormones estrogen and progesterone, and the clinical and laboratory evidence for their involvement are cited. Finally, the developing literature pertaining to various growth factors as the effectors of estrogen and progesterone-induced stimulation is discussed.

Risk Factors Associated with Leiomyomas

Although we have considered and discussed these risk factors, or predisposing factors, in isolation, there is in fact often an overlap or interaction between one or more, for example, obesity, diet, and exercise (Table 1). Second, we can only speculate upon the mechanistic link between these risk factors and fibroid tumorigenesis. Although the impact of many of these factors has often been attributed to their effects upon estrogen and progesterone levels or metabolism, proving this association is difficult, and other mechanisms may well be involved. Finally, there are limitations to the analysis of risk factors, as few epidemiologic studies have been conducted, and reports can easily be biased because of the high prevalence of asymptomatic cases (Schwartz and Marshall 2000).

Menarche

There is a suggestion of slightly increased risk of fibroids associated with early menarche, although the risk has often not been statistically significant (Cramer et al. 1995; Parazzini et al. 1988; Samadi et al. 1996). Recently, a significant inverse association between risk of fibroids and age at menarche was reported; that is, compared with women who were 12 years of age at menarche, those who were ≤ 10 years of age at menarche were at increased risk [relative risk (RR) 1.24], whereas women who were age ≥ 16 years of age at menarche were at lower risk (RR 0.68) (Marshall et al. 1998a). Sato et al. (2000b) found that women with uterine leiomyomas more often exhibited an early normal menstrual cycle pattern, and concluded that early menstrual regularity may enhance leiomyoma growth in early reproductive life. The early onset of menstrual cycles may increase the number of cell divisions that the myometrium undergoes during the reproductive years, resulting in an increased chance of mutation in genes controlling myometrial proliferation (Marshall et al. 1998a).

Parity

Several studies have shown an inverse relationship between parity and the risk of fibroids (Lumbiganon et al. 1996; Parazzini et al. 1996a; Ross et al. 1986; Samadi et al. 1996). A relative risk of fibroids among parous women of 0.5, compared with nulliparae (Parazzini et al. 1988), and a progressive decline in risk relative to the number of births have been reported (Lumbiganon et al. 1996; Marshall et al. 1998a; Parazzini et al. 1996a; Ross et al. 1986; Sato et al. 2000a). An explanation that has been sometimes cited in the literature (Parazzini et al. 1996a; Ross et al. 1986) for these findings is that pregnancy reduces the

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time of exposure to unopposed estrogens, whereas nulliparity or reduced fertility may be associated with anovulatory cycles characterized by long-term unopposed estrogens. The alternative possibility exists that uterine fibroids are actually the cause of the infertility, rather than the consequence of it; however, the diminished relative risk of fibroids associated with parity remains essentially the same after exclusion of women with a history of infertility (Marshall et al. 1998a).

Age

An increase with age in the prevalence of fibroids during the reproductive years has been demonstrated by several epidemiologic studies (Marshall et al. 1997; Ross et al. 1986; Velebil et al. 1995; Wilcox et al. 1994). Studies that define cases by pathologic diagnosis, thus restricting cases to those having surgery (Ross et al. 1986), have shown a rapid increase in fibroid diagnoses among women in their forties. Whether the risk of new fibroids actually increases rapidly in women during their forties is not known. The observed increase could also result from increased growth of, or increased symptomatology from, already existing fibroids, as well as from a greater willingness of women in the later reproductive years to have gynecologic surgery. If the likelihood of fibroid development and growth actually accelerates during the late reproductive years, hormonal factors associated with perimenopause may be important modulators; alternatively, the apparent increase in the late reproductive years may simply represent the cumulative culmination of 20–30 years of stimulation by estrogen and progesterone.

Menopause

A reduced risk of fibroids requiring surgery in postmenopausal patients (Parazzini et al. 1988; Ross et al. 1986; Samadi et al. 1996) could be due to tumor shrinkage in the absence of hormonal stimulus following the menopause. Sectioning of uteri at 2-mm intervals revealed a similar incidence of leiomyomas in pre- and postmenopausal patients (74 and 84%, respectively) although the postmenopausal leiomyomas were smaller and fewer (Cramer and Patel 1990). The estimated risk in postmenopausal patients could be reduced by selection bias because of a tendency toward a more conservative nonsurgical, clinical approach in postmenopausal women (Parazzini et al. 1988).

Obesity

Several studies have found an association between obesity and an increased incidence of uterine leiomyomas. In a prospective study from Great Britain (Ross et al. 1986), the risk of fibroids increased approximately 21% for each 10-kg increase in body weight; similar results were obtained when the body mass index (BMI) was analyzed rather than weight. In a case–control study from Thailand (Lumbiganon et al. 1996), a 6% increase in risk was observed for each unit increase in BMI. Similarly, a large prospective study of registered nurses in the United States (Marshall et al. 1998b) found an increased fibroid risk with increasing adult BMI, as well as an increased risk associated with weight gain since age 18 years. A case–control study from Japan (Sato et al. 1998) likewise reported that women with occult obesity (BMI < 24.0 and percent body fat ≥ 30%) or women with upper-body fat distribution (> 0.80 waist-to-hip ratio) were at significantly higher risk. In a study from Boston, Massachusetts (Shikora et al. 1991), 51% of the hysterectomy- or myomectomy-confirmed patients with leiomyomata were overweight, and 16% were severely obese; the authors compared their patients with a national study group of women in the United States included in The National Health and Nutrition Survey (Abraham and Johnson 1980; Flegal et al. 1998; Van Itallie 1985), quoting comparison figures of 25% overweight and 7.2% severely obese. However, it should be noted that the latter study (Shikora et al. 1991) had no control group of its own, used the percent of desirable body weight as the yardstick rather than BMI, and included fibroid patients from a slightly later time period when the prevalence of obesity was increasing generally in the United States. In contrast to these studies, there are two reports (Parazzini et al. 1988; Samadi et al. 1996) in which no association was found between the incidence of leiomyomas and BMI. Disparate reports of overweight prevalence may relate to definitional criteria, the method of measurement, and choice of comparison groups (Troiano and Flegal 1999).

This apparent association between obesity and an increased risk of fibroids may be related to hormonal factors associated with obesity, but other pathologic pathways might also be involved. Several relevant hormonal associations with obesity are known. A significant increase occurs in the conversion of circulating adrenal androgens to estrone by excess adipose tissue. The hepatic production of sex hormone–binding globulin is decreased, resulting in more unbound physiologically active estrogen. Because almost all circulating estrogens postmenopausally are derived from metabolism of circulating androgens by peripheral tissues, including fat, these two mechanisms probably have more impact in postmenopausal than premenopausal women (Glass 1989). In obese premenopausal women, decreased metabolism of estradiol by the 2-hydroxylation route reduces the conversion of estradiol to inactive metabolites, which could result in a relatively hyperestrogenic state (Schneider et al. 1983).

Diet

The potential role of diet in the genesis of fibroids has received little attention in the literature. In a case–control study in Italy (Chiaffarino et al. 1999), a moderate association was found between the risk of uterine myomas and the consumption of beef, other red meat, and ham, whereas a high intake of green vegetables seemed to have a protective effect. Unfortunately, no estimate of the total caloric intake was obtained, and no attempt was made to estimate the amount of fat in the diet for cases and controls, although one might assume that a higher intake of beef would be associated with a greater amount of fat in the diet. Despite the limitations of the study, the results are interesting and raise a number of issues. Because fibroids are known to be hormonally responsive tumors, are the dietary risks noted above (Chiaffarino et al. 1999) secondary to the effects of various food groups upon the bioavailability of estrogen or progesterone? Is the protective effect of a high intake of green vegetables related to the fiber, some other undetermined component, such as a vitamin, or a corresponding reduction of fat in the diet? What role, if any, do phytoestrogens play?

In a study of premenopausal vegetarian and nonvegetarian women (Goldin et al. 1982; Gorbach and Goldin 1987), the vegetarians

Table 1. Risk factors associated with leiomyomas.

Factor	Risk	Reference
Early menarche	Increased	Marshall et al. 1998a
Nulliparity	Increased	Parazzini et al. 1996a
Age (late reproductive years)	Increased	Marshall et al. 1997
Obesity	Increased	Ross et al. 1986
African-American ethnicity	Increased	Baird et al. 1998
Tamoxifen	Increased	Deligdisch 2000
Increasing parity	Decreased	Lumbiganon et al. 1996
Menopause	Decreased	Samadi et al. 1996
Smoking	Decreased	Parazzini et al. 1996b
Oral contraceptives	?	Marshall et al. 1998a
Hormone replacement therapy	?	Schwartz et al. 1996
Dietary factors	?	Chiaffarino et al. 1999
Xenoestrogens	?	Saxena et al. 1987
Geographic	?	Ezem and Otubu 1981

excreted 3-fold more estrogen in their feces, had lower urinary estrogen excretion, and exhibited 15–20% reduced plasma estrogen levels. This reduction is apparently related to the increased fecal excretion of the estrogen fraction normally excreted in the bile, resulting in diminished enterohepatic circulation of estrogens. There are several possible explanations for the greater fecal excretion of estrogens in vegetarians, including *a*) the greater bulk of undigested and nonabsorbed fiber that may shield the estrogens from bacterial deconjugation and reabsorption; *b*) some characteristic of the vegetarian diet that decreases the ability of the intestinal flora to deconjugate biliary estrogen conjugates, a necessary step for their reabsorption; or *c*) an effect related to lower dietary fat levels that might diminish estrogen absorption. In Goldin's study (Goldin et al. 1982), the vegetarians consumed less total fat and more dietary fiber than did the omnivores. Rose et al. demonstrated that both high-fiber diets (Rose et al. 1991) and low-fat diets (Rose et al. 1987) will reduce serum estrogen levels, probably by altering the fecal flora and reducing the enterohepatic circulation of estrogens. Regardless of the relative importance of dietary fat and fiber, such studies have established that modulation of the diet can influence estrogen metabolism in premenopausal women, which may in turn influence the risk for fibroids. Likewise, a 17% reduction in plasma estradiol concentration was accomplished in postmenopausal women who participated in a low-fat diet intervention program (Prentice et al. 1990).

In recent years plant derivatives known as phytoestrogens have gained attention in both the lay and scientific press. Phytoestrogens are diphenolic compounds that become converted into estrogenic substances in the gastrointestinal tract (Ginsburg and Prelevic 2000). Although these compounds are present in some 300 plants, the quantities present in most are trivial compared with the concentrations in soy and flax; in most populations the major dietary source of phytoestrogens is thought to be soy (Tham et al. 1998). These substances generally act as weak estrogens, but they may also have antiestrogenic effects, depending upon their concentration, the concentration of endogenous estrogens, and individual characteristics such as gender and menopausal status (Ginsburg and Prelevic 2000; Tham et al. 1998); in addition, the effect is probably not identical in different organs (Adlercreutz and Mazur 1997). In this regard, some investigators have suggested that phytoestrogens may act as "natural" selective estrogen receptor (ER) modulators (SERMs, such as tamoxifen) (Ginsburg and Prelevic 2000; Nikov et al. 2000). The observed antiestrogenic effects of phytoestrogens may be partially explained by their competition with endogenous estradiol for ERs (Abramowicz 2000). Prediction of the effects of phytoestrogens is uncertain because there are so many variables involved. Despite their weak estrogenic activity, however, phytoestrogens could conceivably have a significant clinical impact, as their concentrations in the body may exceed those of the endogenous estrogens (Adlercreutz et al. 1982).

Exercise

The possibility of a relationship between exercise and the occurrence of fibroids has been addressed by comparing prevalences among a large group of former college athletes and nonathletes (Wyshak et al. 1986). Former nonathletes were found to be 1.4 times more likely than former athletes to develop benign uterine tumors. In addition to differences in the degree of physical activity, however, an athletic lifestyle may have been associated with long-term differences in diet and relative leanness and, in turn, with reduced conversion of androgens to estrogens in adipose tissue (Frisch et al. 1985; Wyshak et al. 1986).

Racial Differences

There has been a general acceptance in the literature that uterine fibroids are more prevalent in black women than white women. The reference often cited is an early study (Witherspoon and Butler 1934) that had reported that 89.9% of the fibroid patients seen at Charity Hospital in New Orleans, Louisiana, were African American, whereas the total gynecologic admissions were only slightly higher among African Americans than whites. Although this disparity has now been substantiated in a few more current studies, the magnitude of the difference has been less than the factor of 3–9 times sometimes cited (Buttram 1986; Vollenhoven et al. 1990). For instance, in one study (Baird et al. 1998), 73% of black women and 48% of white women had uterine fibroids by ultrasound examination. In a study of hysterectomy specimens, (Kjerulff et al. 1996), 89% of the black women and 59% of the white women had leiomyomas, which in black women were often larger, more numerous, and more symptomatic, and had developed at a younger age. In a recent report (Marshall et al. 1997), 95,061 premenopausal nurses with no history of uterine leiomyoma were followed prospectively and had an incidence rate of leiomyoma approximately 2–3 times greater among black women than among white women. Although there was a higher prevalence of risk factors, including a higher mean BMI, among black women in this latter study, these factors could not account for the excessive rate of uterine leiomyomata among premenopausal black women.

Although the basis for the higher prevalence among black women is unknown, ethnic differences have been found in circulating estrogen levels while on control diets, and differences in estrogen metabolism have been noted. In control groups of healthy, premenopausal women placed on a high-fat, low-fiber diet similar to their usual diet, African-American women had significantly higher serum levels of estrone, estradiol, and free estradiol than Caucasian women. When subsequently placed on a low-fat, high-fiber diet, both groups responded with a significant lowering of their estrogen levels (Woods et al. 1996). In addition, significantly lower 2-hydroxyestrone (2-OHE1)/16α-hydroxyestrone (16α-OHE1) urinary metabolite ratios have been found in African-American women than in Caucasian women (Taioli et al. 1996), which could also contribute to greater estrogen exposure, as 2-OHE1 metabolites are devoid of peripheral biologic activity, whereas 16α-OHE1 is estrogenic. Whether the difference in estrogen metabolism might be due to genetic or environmental factors is unknown.

Fewer data are available regarding the prevalence of uterine fibroids in Hispanics and Asians. In a study of premenopausal nurses in the United States (Marshall et al. 1997), the incidence rates among these two groups, determined by ultrasound or hysterectomy, were similar to those of the white women (rate per 1,000 woman-years = Hispanic 14.5, Asian 10.4, white 12.5, in contrast to black 37.9).

In summary, we conclude that the prevalence of myomas is high among both blacks and whites, and probably also high among Hispanics and Asians, in the United States. The prevalence is relatively higher among African Americans than other ethnic groups based upon ultrasound data, and, more importantly, the clinical prevalence (symptomatic cases) is higher among African Americans because of a higher frequency of multiple lesions and greater size of the fibroids (Baird et al. 1998; Marshall et al. 1997). The issue of clinical prevalence versus total prevalence is an important distinction from an etiologic standpoint, as it indicates that the initiating causes of fibroids may require consideration separate from those factors that could promote their growth to clinically significant proportions.

Geographic Differences

Knowledge of the prevalence of uterine fibroids in other countries could provide clues to the importance of diet, environmental factors, and ethnicity, but unfortunately, few such studies exist in the literature. Sato et al. (Sato et al. 2000b) in Japan stated that "uterine leiomyomas are the most common pelvic tumors" but provided no data of the actual prevalence among their patients. Others (Ezem and Otubu 1981) have cited a 68% incidence of uterine fibromyomata among their hysterectomy cases in Nigeria. A study from Malaysia (Ravindran and Kumaraguruparan 1998) listed fibroids as the main indication for hysterectomy in their

series (47.6% of cases). Similarly, other investigators have implicated fibroid uterus as the main indication for hysterectomy in northern France (66.7% of cases) (Debodinance 2001).

Although no firm statistical conclusions can be drawn, these reports suggest that uterine fibroids occur commonly in women in many parts of the world.

Smoking

Several studies have revealed a reduced risk of fibroids associated with current smoking, but not past smoking (Lumbiganon et al. 1996; Parazzini et al. 1988; Parazzini et al. 1996b; Ross et al. 1986; Samadi et al. 1996; Wyshak et al. 1986). In one study current smokers had a 50% reduced risk of uterine myomas requiring surgery (Parazzini et al. 1996b). In another (Ross et al. 1986) the reduction in risk among smokers was dose dependent; women who smoked 10 cigarettes per day had an 18% decreased risk compared with nonsmokers, whereas smokers of 20 cigarettes per day had a risk approximately 33% lower than that of nonsmokers. In contrast to these results, another survey (Marshall et al. 1998b) found no indication of reduced risk in smokers.

The inverse correlation between smoking and fibroids has been commonly attributed to an antiestrogenic effect of cigarette smoking, suggested by other epidemiologic associations of smoking, including a reduced risk of endometrial cancer, earlier natural menopause, and increased osteoporosis. The pathophysiology of this apparent antiestrogenic effect is not entirely clear, however, because the levels of estrone and total estradiol are often similar in postmenopausal smokers and nonsmokers (Baron et al. 1990), and investigation of hormonal levels in premenopausal smokers has yielded inconsistent results (Longcope and Johnston 1988; MacMahon et al. 1982; Westhoff et al. 1996; Zumoff et al. 1990). On the other hand, several derangements of steroid metabolism have been identified in smokers. Increased 2-hydroxylation of estradiol occurs in smokers, resulting in decreased bioavailability at estrogen target tissues (Michnovicz et al. 1986). Nicotine inhibition of aromatase reduces the conversion of androgens to estrone (Barbieri et al. 1986). Significantly higher serum levels of sex hormone–binding globulin have been found, resulting in less unbound physiologically active estrogen (Daniel et al. 1992). Increased androstenedione and cortisol levels have been noted in postmenopausal smokers, suggestive of increased adrenal activity; elevated androgens may be significant, as some evidence exists that androgens can inhibit estrogen-mediated effects in the rat uterus (Cassidenti et al. 1992; Hung and Gibbons 1983). These studies indicate that the hormonal metabolic effects of smoking are probably multifactorial. In addition, smokers as a group consistently exhibit lower body weights than nonsmokers, possibly because of a lower efficiency of calorie storage and/or an increased metabolic rate (Wack and Rodin 1982). A lower body weight associated with a reduced risk of fibroids might be expected to be another indirect contributing mechanism through which smoking exerts an effect, but in three studies (Lumbiganon et al. 1996; Parazzini et al. 1996b; Ross et al. 1986), the effect of smoking was not changed by correction for BMI (Schwartz et al. 2000a).

Oral Contraceptives

Reports in the literature present inconsistencies with regard to the effect of oral contraceptive (OC) use upon the growth of myomas. An early report suggested that OCs may play a role in the development or growth of leiomyomata (John and Martin 1971). Some have found no association between the occurrence of fibroids and the use of OCs (Parazzini et al. 1992; Samadi et al. 1996); however, others have reported a reduction in risk of fibroids with OC use (Ratech and Stewart 1982; Ross et al. 1986). Further, in the study by Ross et al., a consistent decrease in the risk of fibroids was noted with increasing duration of OC use (approximate 17% reduction in risk with each 5 years of use); this apparent protective effect was attributed to reduced exposure to unopposed estrogen due to the modifying effect of progestogens (Ross et al. 1986). This study was criticized, however, for indication bias (Ratner 1986), as fibroids had commonly been considered a contraindication to OC use, thus resulting in a selected group for study.

These conflicting findings with regard to the effect of OCs upon the growth of myomas may relate to the differing content of estrogen and the type of progestogen in each specific OC preparation (Cramer 1992). In fact, Ross et al. (1986) attempted to address this issue by analyzing the estrogen and progesterone content of each formulation. Although no conclusions could be drawn regarding the estrogens present, the authors found that the higher the dose of the progestogen norethisterone acetate, the lower the incidence of fibroids, in preparations containing the same quantity of the estrogen ethinylestradiol. In contrast, all preparations containing the progestogen ethynodiol diacetate were associated with an increased incidence of fibroids, regardless of the quantity present or the type or amount of the accompanying estrogen. The authors offered no explanation for the latter finding and stated that additional studies were needed for confirmation.

A significantly elevated risk of fibroids has been reported among women who first used OCs in their early teenage years (13–16 years of age) compared with those who had never used them (Marshall et al. 1998a).

Hormone Replacement Therapy

Fibroids are expected to shrink after menopause, but hormone replacement therapy (HRT) may prevent this shrinkage and may even stimulate growth. Two studies that were conducted when estrogen was prescribed without progestins reported elevated risk of fibroid surgery (Romieu et al. 1991) or uterine leiomyomata requiring hospitalization (Ramcharan et al. 1981) among women taking HRT. Addition of progestins does not appear to reduce risk. One large (Polatti et al. 2000) and several small (Colacurci et al. 2000; Fedele et al. 2000; Sener et al. 1996; Ylostalo et al. 1996) clinical trials demonstrated increased fibroid size during treatment with transdermal estrogen when progesterone was included. Similarly, injected estrogen plus progestin resulted in an increase in the size and number of myomas (Frigo et al. 1995). On the other hand, in four studies (Clark and Johnson 2000; de Aloysio et al. 1998; Polatti et al. 2000; Sener et al. 1996) using oral HRT, little change in tumor size was noted. In another investigation oral HRT (using a heterogeneous variety of treatment regimens including two estradiol-patch patients) was accompanied by an increase in volume of 17 myomas and a decrease in size of 6 myomas, but the changes were not statistically significant (Schwartz et al. 1996). Several of the oral HRT studies did not include a control group of postmenopausal women who were not on HRT; however, in the two reports that did include control groups (Clark and Johnson 2000; Schwartz et al. 1996), the myoma volume decreased over time in the control group, although not significantly in one study (Schwartz et al. 1996). Taken together, these studies suggest that oral HRT may not stimulate the growth of myomas or may result in growth of some but not other myomas. Although little data are available, the two studies with control groups (Clark and Johnson 2000; Schwartz et al. 1996) suggest that oral HRT may inhibit normal menopausal regression of fibroids.

The effect of HRT on fibroids in postmenopausal women is obviously a complicated issue resolvable only by future well-controlled studies. Further emphasizing this point is the assertion (Polatti et al. 2000) that an increase in volume or number of uterine myomas during HRT in postmenopause is likely not related solely to the dose and route of administration of the estrogen, but also to the type and dosage of progestogen.

Tamoxifen

Tamoxifen is a partial estrogen agonist that binds to ERs in receptive cells, thereby antagonizing the effects of estrogen by competitively

binding to target organ receptors. Because tamoxifen is effective adjuvant therapy for ER-positive breast cancer, it might be expected to induce regression of estrogen-responsive uterine fibroids. Indeed, there are *in vitro* studies indicating that tamoxifen does inhibit estrogen-stimulated growth of Eker rat–derived uterine leiomyoma cell lines (Fuchs-Young et al. 1996). However, several clinical studies have now reported the growth or enlargement of uterine fibroids in breast cancer patients undergoing tamoxifen therapy. In some cases the expansion of tumor volume has been sufficiently great to require hysterectomy. Although these reports are anecdotal, several have included postmenopausal patients in whom fibroids typically regress rather than enlarge. On the other hand, if tamoxifen were efficacious in shrinking the size of fibroids in some patients, one might expect to see anecdotal reports of such, but we were unable to find any in the literature. These clinical reports collectively seem to indicate that the *in vivo* effect of tamoxifen, in both pre- and postmenopausal patients at the dosage levels ordinarily used as therapy in breast cancer patients, is either to stimulate the growth of uterine fibroids or to exert no effect (Boudouris et al. 1989; Dilts et al. 1991; Kang et al. 1996; Le Bouedec et al. 1995; Leo et al. 1994; Lumsden et al. 1989a; Tomas et al. 1995; Ugwumadu and Harding 1994). In a recent review (Deligdisch 2000), tamoxifen for breast carcinoma was reported to exert an estrogen-agonist effect on the uterus in approximately 20% of patients, who developed endometrial polyps, glandular hyperplasia, adenomyosis, and/or leiomyomata. A few cases of uterine leiomyosarcoma developing in patients on tamoxifen therapy have also been reported (Chew et al. 1996; Kennedy et al. 1999; McCluggage et al. 1996; Sabatini et al. 1999; Silva et al. 1994). This apparent estrogenic agonist effect of tamoxifen is further supported by the lack of shrinkage of uterine leiomyomas by gonadotropin-releasing hormone (GnRH) agonists when used in combination with tamoxifen (Lumsden et al. 1989b).

Several inferences may be drawn from these reports. First, the biologic actions of tamoxifen are complex, and the information gained from animal models and tissue culture is not necessarily directly transferable to humans. Second, the disparate effects of tamoxifen in the breast and uterus exemplify the mixed agonist/antagonist activity of SERMs, which is apparently dictated by the cell type and the promoter context of the ERs for a given cell type (Hall et al. 2001).

Xenoestrogens

A diverse group of exogenous compounds, xenoestrogens, possesses the potential to disrupt normal estrogenic function as a result of either estrogenic agonist or antagonistic effects. No common chemical structure is predictive of estrogenic activity, and such substances may originate from dietary, industrial, or pharmaceutical sources (Houston et al. 2001). Although industrial chemicals with estrogenic effects have come under recent scrutiny, few studies have specifically addressed this issue in regard to fibroid tumorigenic effects, despite the known sensitivity of uterine leiomyomas to estrogenic stimulation (Hunter et al. 2000).

The pesticide dichlorodiphenyltrichloroethane (DDT) and its analogs have been shown to be estrogenic (Cecil et al. 1971). Although banned in this country for more than two decades, residues of organochlorine pesticides remain detectable in mammalian fat stores (Stellman et al. 1998), and some DDT analogs such as methoxychlor are still in common use in the United States (Meadows 1996). In the only human studies, to our knowledge, of DDT and uterine fibroids (Saxena et al. 1987), significantly higher levels of DDT and its metabolites were found in uterine leiomyomatous tissue than in normal myometrium, and significantly higher levels of DDT were reported in the blood of women with uterine leiomyomas than in those without (Khare 1985). In *in vitro* studies with Eker rat uterine leiomyoma–derived cells, several organochlorine pesticides, including 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane, kepone, endosulfan-α, methoxychlor, dieldrin, toxaphene, and endosulfan-β acted as ER agonists, upregulating progesterone receptor expression and in some cases stimulating proliferation of leiomyoma cells (Hodges et al. 2000). Further, the mobilization of organochlorines (stored in mammalian fat) that occurs during lactation (Sonawane 1995) and fasting (Bigsby et al. 1997) could result in exposure levels severalfold higher than those originally encountered in the environment (Hodges et al. 2000). Also of interest is the finding that the more recently recognized ER-β binds two xenoestrogens, methoxychlor and bisphenol A, with considerably higher affinity than the classic ER, ER-α (Enmark et al. 1997). In view of the widespread use and exposure to the organochlorine pesticides and other environmental estrogens, a need clearly exists for further investigation of a possible link to fibroid pathogenesis. Studies with the potent synthetic estrogen diethylstilbestrol have clearly indicated that exogenous estrogen exposure during critical stages of development can result in permanent cellular and molecular alterations (Newbold 1995), including the formation of uterine leiomyomas (Newbold et al. 2002).

Initiators of Tumorigenesis

Theories of Initiation

The most important aspect of the etiology of fibroids—the initiator(s)—remains unknown. Several theories have been advanced. One hypothesis states that increased levels of estrogen and progesterone result in an increased mitotic rate that may contribute to myoma formation by increasing the likelihood of somatic mutations (Rein 2000). Another favors an inherent abnormality in the myometrium of those who develop fibroids, based upon the finding of significantly increased levels of ER in the myometrium of fibroid uteri (Richards and Tiltman 1996). A predisposing genetic factor has been suggested by others on the basis of ethnic and familial predilections (Marshall et al. 1997; Schwartz et al. 2000b).

Another interesting theory postulates that the pathogenesis of uterine leiomyomas might be similar to a response to injury (Stewart and Nowak 1998) in a manner analogous to the development of keloids (hypertrophic scars) following surgery. One avenue of potential injury might be ischemia associated with the release of increased vasoconstrictive substances at the time of menses. Increased secretion of prostaglandins and vasopressin by the endometrium has been noted in patients with dysmenorrhea (Emans et al. 1998), which occurs in up to 70% of women by the fifth year after menarche (Coupey 2000). Might the smooth muscle cells of the myometrium react to injury in a manner analogous to vascular smooth muscle cells by undergoing a transformation from a contractile phenotype to a proliferative-synthetic phenotype? Certainly, morphologic similarities exist, as fibroids exhibit both an increased proliferative rate (Dixon et al. 2002) and the synthesis of extracellular fibrous matrix. After vascular injury, basic fibroblast growth factor (bFGF) is critical to smooth muscle proliferation, and this factor is also overexpressed in leiomyomas (Lindner and Reidy 1991; Mangrulkar et al. 1995). Finally, injury related to menses is worthy of consideration in view of the universality of menstruation and the commonality of fibroids. When we consider the various risk factors, including those that have been attributed in the literature to increased exposure to "unopposed estrogens," such as early menarche and nulliparity, we observe that such patients also experience more menstrual cycles than their counterparts.

Of equal uncertainty in the genesis of fibroids is the role of genetic and/or epigenetic changes. The possibility of hereditary genetic predisposition to fibroids cannot be excluded at this time. On the other hand, evidence has been presented, though limited in scope, that karyotypic changes may occur secondarily (Mashal et al. 1994) during the evolution or

aging of some fibroids. Regardless of whether acquired karyotypic changes occur *ab initio* or during clonal evolution of fibroids, we can assume that preceding stimuli, conditions, or injuries must be responsible for the induction of genetic or epigenetic changes, and in this sense acquired genetic changes may be regarded as secondary. These changes are therefore discussed in this section, not from the standpoint of purported initiators, but as possible potentiators or effectors of currently unrecognized initiating conditions.

The Genetic Findings

There have been numerous studies and reviews of the clonality and cytogenetics of uterine leiomyomas (Gross and Morton 2001; Ligon and Morton 2000, 2001; Mark et al. 1988; Nilbert and Heim 1990; Ozisik et al. 1993b; Pandis et al. 1991). For the purposes of this brief review, we have attempted to summarize those features that appear most salient.

Heritability. Is there evidence of a genetic predisposition to fibroids? This question has been approached from four perspectives: ethnic predisposition, twin studies, familial aggregation, and association with an inherited syndrome. The higher incidence of clinically significant fibroids among African-American women in the United States has been discussed above. Two studies comparing monozygous and dizygous twins may be cited. The first of these reported a 2-fold higher correlation for hysterectomy in monozygotic than dizygotic twins (Treloar et al. 1992). Because leiomyomata represent the most common indication for hysterectomy in the United States, this finding in monozygous twins suggests a genetic liability for fibroids. Because the study did not report the actual incidence of leiomyomata, however, it is recognized that heritable conditions other than fibroids could contribute to the observed correlation in twins (Gross and Morton 2001). A more recent twin study specifically addressed the risk of fibroids in twins by examining hospital discharge diagnoses from the Finnish Twin Cohort Study and by performing transvaginal ultrasounds in a random sample of these women (Luoto et al. 2000). The casewise concordance for hospitalization due to uterine fibroids was significantly higher in monozygous twins than dizygous twins, providing support for a genetic contribution. On the other hand, by ultrasound examination the risk ratio for fibroids in a monozygous twin whose sister had been diagnosed with fibroids was only 1.1, the same as for a dizygous twin; however, the authors noted that the low participation rate decreased the power of the study to detect potential differences between the twins. The study concluded that anthropometric and reproductive factors, such as a higher BMI and nulliparity, may play at least as large a role in pathogenesis of fibroids as genetic factors.

Four studies of the familial clustering of fibroids may be cited. The first was a German study, reported in 1938 (Winkler and Hoffmann 1938), in which fibroids were found to be 4.2 times more common in first-degree relatives of women with fibroids than those without. Similar findings were noted in two studies from Russia in which a higher incidence of fibroids was found in first-degree relatives (Vikhlyaeva et al. 1995) and sisters (Kurbanova et al. 1989) of affected probands than in controls. Last, in a study of 638 fibroid patients and 617 controls in the Puget Sound area of Washington State (Schwartz et al. 2000b), fibroid patients again were found more likely than the controls to report a history of fibroids in a mother or sister (33.2% vs. 17.6%). Furthermore, the odds ratio increased to 5.7 in cases of early-onset fibroids, as might be expected for a genetically influenced trait. Unfortunately, these studies may be influenced by reporting and detection bias. A woman having clinical problems that could be attributed to fibroids may be more likely to seek a diagnosis if a close relative has had fibroids. A woman who has been diagnosed may also be more likely to learn about diagnoses among her female relatives.

Finally, a rare inherited disorder known as Reed's Syndrome (Fisher and Helwig 1963; Reed et al. 1973; Thyresson and Su 1981), or multiple leiomyomatosis, is characterized by the appearance of multiple leiomyomas in the skin, uterus, or both. The family histories in these cases suggest an autosomal dominant inheritance with incomplete penetrance. Recent reports of several families in England and Finland with multiple uterine and cutaneous leiomyomata, and a subset of these with papillary renal cell carcinoma, have independently linked this disorder to a predisposition gene in the region of chromosome 1q42.3-q43 (Alam et al. 2001; Kiuru et al. 2001; Launonen et al. 2001). In follow-up studies of this chromosomal region, mutations were detected only in the fumarate hydratase gene (Tomlinson et al. 2002)—a surprising finding, as this enzyme is a component of the essential energy-producing tricarboxylic acid cycle (Rustin et al. 1997). Furthermore, the gene appears to act as a classic tumor suppressor in that loss of the wildtype allele was observed frequently in the leiomyomata and renal cell cancers (Alam et al. 2001; Kiuru et al. 2001; Launonen et al. 2001). Although this hereditary syndrome is itself rare, the association with inactivation of the fumarate hydratase gene is of interest, as it is possible that other mechanisms of transcriptional silencing of this gene such as promoter hypermethylation could be involved in the development of sporadic leiomyomas (Kiuru et al. 2001).

Clonality. There is general acceptance in the literature that these tumors are monoclonal. The underlying premise of these studies has been based on the Lyon hypothesis, which assumes that only one X chromosome is active in any female cell, the other X chromosome remaining in an inactive state as a Barr body, and that the X chromosome that is inactivated (methylated) is determined randomly. Thus, genetic loci known to be located on the X chromosome can be studied in these tumors for evidence of homogeneity of expression in those patients identified as heterozygous for a particular gene in their normal, nontumor tissues.

The first studies of clonality used the X-linked glucose 6-phosphate dehydrogenase (G6PD) isozymes. After screening patients for G6PD heterozygosity by analysis of red blood cells, the resected fibroids and myometrium were analyzed for the presence of one or both electrophoretic types of G6PD. In two studies (Linder and Gartler 1965; Townsend et al. 1970), both G6PD types (A and B) were identified in almost all samples of myometrium, whereas only one G6PD type (A or B) was identified in each of the leiomyomas. Furthermore, both A and B tumors were often identified in the same patient, indicating independent origins of the individual fibroids. These results suggested that the tumors arose from single cells, although selective overgrowth of one cell type from a tumor originally composed of both G6PD types cannot be excluded. The major limitation of these studies is the minor degree of G6PD polymorphism in the population, as most Caucasian females (> 99%) are homozygous type B, and only 30% of African-American females are heterozygous, and therefore only a minority of cases would be informative by studies of this gene.

More recently, clonality studies have taken advantage of methylation-sensitive restriction enzymes to discriminate between active and inactive alleles of X-linked genes known to be highly polymorphic (Vogelstein et al. 1985). Tumors arising from single cells should contain only one type of inactive (methylated) allele, which will be amplified exclusively following restriction-enzyme digestion of the active (unmethylated) allele, whereas tumors of multicellular origin should contain some cells with one type of inactive allele and other cells with a second type of inactive allele, resulting in the amplification of both alleles following digestion and polymerase chain reaction. This method has been employed for analysis of both the X-linked androgen receptor gene (Mashal et al. 1994) and the X-linked phosphoglycerokinase gene (Hashimoto et al. 1995). Both studies concluded that the uterine fibroids examined were monoclonal in origin.

One report has described chromosome 7 biclonality in four uterine leiomyomas (Ozisik et al. 1993a), with the breakpoint regions in two of these such that one clone could not possibly have originated from the other clone. Taken in sum, however, the concept of monoclonal origin of most fibroids appears to be a valid one, recognizing that some could be biclonal in origin (Ozisik et al. 1993a) and some are biclonal or oligoclonal because of clonal evolution (Pandis et al. 1990), and that monoclonality itself could be the result of selective overgrowth of one clone from an originally polyclonal proliferation (Fey et al. 1992; Vogelstein et al. 1987).

Cytogenetics. Most of the investigations of leiomyomas seeking chromosomal aberrations have used classic cytogenetic karyotyping, a valuable tool because it is the only method that allows one to survey the entire genetic constitution of a tissue with a single assay. Standard cytogenetic methodology with G-band analysis can identify translocations, deletions, and duplications, but does require the *in vitro* culture of leiomyoma cells to obtain metaphase preparations. An alternative method that has been employed in a few studies (Levy et al. 2000; Packenham et al. 1997) is comparative genomic hybridization, which permits the recognition of cytogenetic changes such as deletions and amplifications without the need for cell cultures of the tumor, although not allowing for detection of balanced rearrangements. Neither standard karyotyping nor comparative genomic hybridization permits the detection of small, submicroscopic chromosomal abnormalities such as point mutations or epigenetic changes such as methylation.

Most common cytogenetic changes. Because the studies of tumor cytogenetics are limited to tissue samples removed at surgery and may be taken from larger fibroids, the possibility exists that they may not be representative of leiomyomas in general. Nonetheless, based upon such samples, approximately 40–50% of uterine fibroids are reported to have nonrandom chromosomal abnormalities (Table 2).

t(12;14). One of the most common of these is a translocation between chromosomes 12 and 14, specifically t(12;14) (q14-q15;q23-q24), which is present in about 20% of karyotypically abnormal leiomyomata (Ligon and Morton 2000). This abnormality is of interest for several reasons. First, the region q14-q15 on chromosome 12 is also commonly rearranged in a variety of other mesenchymal solid tumors, including angiomyxomas, hemangiopericytomas, lipomas, and pulmonary chondroid hamartomas, as well as breast fibroadenomas, endometrial polyps, and salivary gland adenomas. In addition, evidence exists that a critical gene located in the chromosome 12q14-q15 region may be *HMGIC* (now designated *HMGA2*), a gene encoding a member of the high-mobility group (HMG) of proteins. These are DNA-binding proteins that can induce conformational changes in DNA, thereby indirectly regulating transcription by influencing the access of other DNA-binding proteins to target genes. The HMGIC protein may play a role as a proliferation factor in growing tissues, particularly those of mesenchymal origin; accordingly, expression of this protein has been detected in leiomyomata with 12q14-15 rearrangements, but not in matched normal myometrium (Gattas et al. 1999). In addition, the region on chromosome 14 involved in this translocation, q23-q24, is of particular interest because of its specificity for fibroids compared with other mesenchymal tumors in which *HMGIC* is rearranged. The ER-β gene (*ESR2*) is located in this region of chromosome 14 and would seem to be a logical fusion partner with *HMGIC*, as the growth of fibroids is responsive to estrogen. More recently, *ESR2* has been mapped to a region approximately 2 Mb centromeric to the t(12;14) breakpoint, suggesting that *ESR2* is not involved with *HMGIC*. However, this finding may not exclude the possibility that *ESR2* might be deregulated by chromosomal translocation in view of its proximity to the breakpoint (Pedeutour et al. 1998).

Evidence has also been presented that *RAD51L1* (formerly *RAD51B*), a member of the *RAD51* recombination repair gene family (Albala et al. 1997; Shinohara et al. 1992), is the chromosome 14 target gene and preferential fusion partner of *HMGIC* in uterine leiomyomas with t(12;14) (Amant et al. 2001; Ingraham et al. 1999; Schoenmakers et al. 1999; Takahashi et al. 2001). Although the RAD51L1 protein has not yet been shown to catalyze recombination reactions, *RAD51L1* appears to be an essential gene (Shu et al. 1999) expressed in almost all organs and tissues (Rice et al. 1997) and probably plays a role in regulation of cell cycle progression (Havre et al. 1998, 2000). In view of the purported function of *HMGIC* in regulation of cell proliferation (Reeves 2000) and the probable role of *RAD51L1* in cell cycle regulation, it is reasonable to speculate that the disruption of genomic structure associated with the *RAD51L1/HMGIC* fusion (Ingraham et al. 1999; Schoenmakers et al. 1999; Takahashi et al. 2001) might result in dysregulated cell growth.

del(7q). Another frequently encountered karyotypic abnormality in fibroids is a deletion of chromosome 7, del(7)(q22q32), which is present in about 17% of karyotypically abnormal fibroids (Ligon and Morton 2000). In some series del(7q) has been the most common cytogenetic abnormality in fibroids (Nilbert and Heim 1990; Ozisik et al. 1993b). Although interstitial deletions and translocations involving chromosome 7q have also been reported in other benign tumors, such as lipomas and endometrial polyps, the deletion is more commonly observed in fibroids than in any other solid tumor. Because this region, 7(q22q32), is physically large and gene-rich, pinpointing a specific gene that could be implicated in the genesis of fibroids has proven difficult. Recently, however, the critical area on band 7q22 has been narrowed to a 4-cM (centiMorgan) region by allelotype analysis (van der Heijden et al. 1998). In the latter study loss of heterozygosity in the leiomyomas was rare except in 7q22, where a minimal deletion was observed in 34% of the tumors, leading the authors to speculate that this site probably harbors a novel tumor-suppressor gene involved in the etiology of this tumor (van der Heijden et al. 1998).

6p21. A third cytogenetic subgroup consists of aberrations of 6p21, including deletions, inversions, translocations, and insertions. Interest in this region has been related in part to the frequently observed alterations of band 6p21 in other benign mesenchymal tumors, such as lipomas, and to the identification of another high mobility group gene, *HMGIY* (now designated *HMGA1*), in this region. Rearrangements of 6p21 are much less common in fibroids than in these other tumors, however, occurring with a frequency of < 5%.

Trisomy 12. A variety of other cytogenetic abnormalities have been identified in leiomyomata. The reporting of trisomy 12 in as many as 12% of karyotypically abnormal fibroids (Nilbert and Heim 1990; Vanni et al. 1992) raises the question of whether this anomaly might reflect pathogenetic similarities to t(12;14), acting to increase the gene dosage of *HMGIC*. Many of the other abnormalities, such as ring chromosomes, occur less frequently and often concomitantly with other chromosomal changes and are therefore thought to represent secondary abnormalities.

Correlations with tumor phenotype. No indication of systematic histologic differences between leiomyomas with normal karyotypes and those with chromosomal aberrations were

Table 2. Leiomyoma-associated cytogenetic changes.

Chromosomal aberration	Frequency (%)[a]	Reference	Gene candidate
t(12;14)(q14-q15;q23-q24)	20	Ligon and Morton 2000	*TGFβ3, HMGIC (HMGA2)*
del(7) (q22-q32)	17	Ligon and Morton 2000	Numerous
Trisomy 12	12	Nilbert and Heim 1990	Numerous
6p21 (del, inv, t, ins)	< 5	Ligon and Morton 2000	*HMGIY (HGMA1)*

[a]Frequency among those leiomyomas with abnormal karyotypes.

found in one study (Nilbert and Heim 1990); however, there is some evidence from other reports (Meloni et al. 1992; Pandis et al. 1990) that leiomyomas that are either cellular with mitotic activity or atypical histologically are more likely to demonstrate karyotypic abnormalities or to show massive karyotypic aberrations indicative of clonal evolution. In a study of 114 myomas from 92 patients, myomas > 6.5 cm demonstrated a significantly higher proportion of abnormal karyotypes than myomas < 6.5 cm (75% vs. 34%) (Rein et al. 1998). In the same study a relationship between particular karyotypes and fibroid size was identified, with the largest tumors carrying t(12;14) abnormalities and the smaller tumors exhibiting chromosome 7 deletions, suggesting that chromosomal abnormalities associated with individual myomas may enhance myoma growth. A correlation between the location of the fibroid and the likelihood of a cytogenetic abnormality has also been reported (Brosens et al. 1998); submucous myomas presented significantly fewer abnormal karyotypes (12%) than did either the intramural (35%) or the subserosal (29%) tumors, and furthermore, this correlation remained significant regardless of the diameter of the myoma.

Summary. Despite the large number of cytogenetic studies, many unanswered questions remain. Are the chromosomal aberrations primary to the genesis of these tumors or are they secondary events? In one study chromosomal abnormalities were interpreted as secondary events because they were preceded by monoclonality (Mashal et al. 1994); however, the data are limited and additional studies are needed for verification. Certain karyotypic abnormalities such as the t(12;14) and the del(7q) occur with sufficient frequency to warrant consideration as differing pathways leading to leiomyoma development, or at least to consider that these sites may contain genes that are important in the proliferation and differentiation of smooth muscle cells. Because at least one-half of fibroid tumors appear to be cytogenetically normal, there may exist an unidentified submicroscopic mutation in this karyotypically normal subgroup or even in the cytogenetically abnormal group as well. Histologic subtypes such as the cellular and atypical leiomyomas may ultimately be correlated with certain karyotypic aberrations that are either distinctive primary events or represent secondary changes of clonal evolution. Finally, regarding heritability, a particular gene or genes may one day be identified as predisposing to the development of leiomyomata, as suggested by the familial clustering studies. If so, it must be a very common gene, widespread in the general population, in view of Cramer and Patel's finding of a 77% incidence of leiomyomas in a thorough examination of 100 consecutive, nonselected hysterectomy specimens (Cramer and Patel 1990).

Promoters: Evidence for the Role of Estrogen and Progesterone

Clinical Observations

Estrogen has been traditionally proposed as the primary promoter of uterine leiomyoma growth. This supposition has been based in part upon the clinical observations that fibroids occur only after menarche, develop during the reproductive years, may enlarge during pregnancy, and frequently regress following menopause. Furthermore, because the risk of fibroids is greater in nulliparous women who might be subject to a higher frequency of anovulatory cycles and obese women with greater aromatization of androgens to estrone in the fat, the concept of unopposed estrogens as an underlying cause of uterine fibroids has sometimes been proposed in the literature (Cramer 1992; Parazzini et al. 1996a; Romieu et al. 1991; Ross et al. 1986). Increased growth of myomas among women taking tamoxifen or receiving transdermal or injected estrogen-replacement therapy further supports the importance of estrogen. The estrogen hypothesis has also been supported by clinical trials evaluating the medical treatment of myomas with GnRH agonists, the effective result of which is hypoestrogenism accompanied by regression of the fibroids (Friedman et al. 1989). As noted by Rein, however, distinguishing the relative importance of estrogen versus progesterone is difficult, as progesterone levels, in a manner similar to those of estrogen, are also cyclically elevated during the reproductive years, are significantly elevated during pregnancy, and are suppressed after menopause (Rein et al. 1995). Furthermore, regression of uterine leiomyomata has been induced by treatment with the antiprogesterone drug RU 486, accompanied by reduction in the progesterone receptor (PR) but not the ER in the tumors, suggesting that the regression was attained through a direct antiprogesterone effect (Murphy et al. 1993). In addition patients treated with leuprolide (a GnRH agonist capable of reducing the size of fibroids) who were concomitantly given medroxyprogesterone acetate demonstrated no significant reduction in myoma or uterine volume (Carr et al. 1993; Friedman et al. 1988). Indeed, clinical and laboratory evidence to date would appear to indicate that estrogen and progesterone may both be important as promoters of myoma growth (Rein 2000).

We now consider further the impact of sex steroids upon fibroid growth in two diametrically opposed clinical situations, namely, pregnancy with the associated elevations of estrogen and progesterone, and medical treatment with GnRH agonists accompanied by reductions in these two hormones.

Pregnancy. A common clinical perception prevails that myomas increase in size during pregnancy (Buttram 1986). With the advent of ultrasonographic studies, however, several reports have noted that only a minority of myomas (one-third or less) increase in size during pregnancy, whereas the majority remain stable or decrease in size (Aharoni et al. 1988; Rosati et al. 1992; Strobelt et al. 1994). The larger the myoma, the greater the likelihood of growth (Strobelt et al. 1994). Myoma size can increase as a result of hypertrophy and edema, while shrinkage of the tumor may occur as a result of degenerative changes secondary to ischemia. A 10% complication rate related to myomas has been reported during pregnancy (Katz et al. 1989). The most common complication was the syndrome of painful myomas, sometimes associated with bleeding, and probably related to hemorrhagic degeneration or infarction. Although the etiology of the syndrome of painful myomas of pregnancy is unclear, high concentrations of progesterone, as in pregnancy, may play a role, as similar changes of "red degeneration" have been induced by high-dosage progestin therapy (Goldzieher et al. 1966). Other reported complications of myomas in pregnancy include premature rupture of the membranes, malpresentation, increased cesarean delivery rate, and postpartum endomyometritis (Katz et al. 1989). It has also been suggested that fibroids are a more important feature in pregnancy now than in the past because many women are delaying childbearing to their late thirties, the time of greatest risk for fibroid growth (Vollenhoven et al. 1990).

Gonadotropin-releasing hormone agonists (luteinizing hormone–releasing hormone agonists). GnRH analogs are therapeutic agents derived from peptide substitutions of the hypothalamic hormone luteinizing hormone–releasing hormone (LHRH). These substitutions at positions 6 and 10 in the amino acid structure result in analogs that are 40–200 times more potent than native LHRH (Vollenhoven et al. 1990). Although the initial response to these agents is an elevation of serum gonadotrophin levels and with it increased concentrations of sex steroids, continuous administration results in suppression of the pituitary–ovarian axis, with decreased gonadotropin and sex steroid levels. The mechanism of this suppression is thought to be related to downregulation of the pituitary LHRH receptors (Fraser 1988). The hypoestrogenic state induced by these agents results in reduction in size of the uterus itself as well as many of the fibroids in the majority of patients. A variety of theories have been proposed for the pathophysiologic mechanism leading to this shrinkage of fibroids, including a reduction in uterine arterial blood flow

(Shaw 1989), a combination of ischemic injury and cellular atrophy (Colgan et al. 1993), a reduction in cellularity (Upadhyaya et al. 1990), apoptosis (Higashijima et al. 1996), and a reduction in the number of cycling cells secondary to reduced levels of ER and PR (Robboy et al. 2000; Vu et al. 1998).

Unfortunately, use of these agents as the sole therapy for fibroids is limited by the rapid enlargement of the myomas to near pretreatment size following cessation of the GnRH agonist therapy (Friedman et al. 1989) and by the concern for potential bone resorption with long-term administration of the drugs (Friedman et al. 1990). However, GnRH analogs have been used as preoperative therapy to reduce the size of fibroids prior to hysterectomy; this approach has resulted in reports of significantly less blood loss at operation (Lumsden et al. 1987) and increased feasibility of vaginal rather than abdominal hysterectomy, accompanied by shorter hospitalizations (Stovall et al. 1991).

Laboratory Studies

Estrogen and progesterone levels. Patients with uterine leiomyomas have plasma estradiol and progesterone levels similar to those of women without detectable myomas, as indicated in three studies (Dawood and Khan-Dawood 1994; Maheux et al. 1986; Spellacy et al. 1972). An older report noted that the urinary estrogens of approximately one-third of the fibroid patients were elevated with respect to their laboratory normal range, but no control group was available for comparison (Timonen and Vaananen 1959). Quantitative differences, however, have been demonstrated between leiomyomas and myometrium in the tissue concentrations of ovarian hormones, their receptors, and a key metabolizing enzyme. In one study, the concentration of 17β-estradiol was significantly higher in leiomyomas than myometrium, especially in the proliferative phase, whereas no difference in the concentration of progesterone was found (Otubu et al. 1982). The authors speculated that the higher levels of estradiol in the leiomyomas could be related to lower levels of the enzyme 17β-hydroxysteroid dehydrogenase, which accelerates the conversion of estradiol to estrone. Other investigators have also demonstrated higher estradiol concentrations (Folkerd et al. 1984) and more frequent expression or overexpression of aromatase activity in leiomyomata than in matched myometrial samples (Folkerd et al. 1984; Sumitani et al. 2000; Yamamoto et al. 1984), leading these authors to entertain the possibility that increased androgen to estrogen conversion in fibroids may potentiate their growth.

Estrogen and progesterone receptors. The ER and PR literature comprises a rather extensive and sometimes contradictory collection of data that spans several decades of research. Disparate results are probably attributable to the diversity of methodologies employed (including assessment of the cytosol alone versus the combined nuclear and cytosolic fractions), the use of human versus nonhuman tissues, the phase of the menstrual cycle at the time of collection of specimens, and the heterogeneity of myomas in the same patient (Englund et al. 1998). In the absence of experimental unanimity, the generalizations or conclusions that follow are therefore based upon our assessment of the weight of the evidence.

In the majority of the studies reviewed, the concentrations of both the ERs and PRs were greater in leiomyomata than the myometrium (Andersen et al. 1995; Brandon et al. 1993, 1995; Buchi and Keller 1983; Eiletz et al. 1980; Englund et al. 1998; Kawaguchi et al. 1991; Lessl et al. 1997; Marugo et al. 1989; Nisolle et al. 1999; Otsuka et al. 1989; Pollow et al. 1978a; Puukka et al. 1976; Rein et al. 1990c; Sadan et al. 1987; Soules and McCarty 1982; Tamaya et al. 1979, 1985; Vij et al. 1990; Viville et al. 1997; Vollenhoven et al. 1994; Wilson et al. 1980). In addition, Sadan et al. found the ER and PR to be elevated in fibroids during all phases of the menstrual cycle when compared with matched myometria (Sadan et al. 1987). Interestingly, in one study (Marugo et al. 1989) the ER and PR levels were significantly higher in submucous than subserosal leiomyomas, leading the authors to speculate about different etiologies and types of leiomyomas. The receptor concentrations were independent of the size of the tumor in one report (Sadan et al. 1987). Another investigation found marked variation in ER and PR levels in different tumors from the same subject (Englund et al. 1998); such heterogeneity may relate to the degree of hyalinization and involution of individual tumors.

ER-α and ER-β. Because a second subtype of the ER, designated ER-β, was not discovered until 1996 (Kuiper et al. 1996; Mosselman et al. 1996), the significance of ER-β relative to that of the classic ER, ER-α, has not been fully determined. Nuclear expression of both ER-α and ER-β throughout the entire myometrium has been demonstrated immunohistochemically (Taylor and Al-Azzawi 2000). One group (Pedeutour et al. 1998) found ER-β mRNA in 14 of 15 leiomyomata, with no striking difference in expression from the matched myometrial tissues. Another group (Benassayag et al. 1999) showed expression of both ER-α and ER-β mRNA in leiomyomata, with the levels of both receptors higher in most of the leiomyomas than in the corresponding nonpregnant myometria. Andersen noted that the highest expression of ER-β in nonpregnant myometrial and leiomyoma tissues is at the beginning of the menstrual cycle, and the lowest expression is at the early midluteal phase; however, low levels of ER-β protein were detected in these tissues, in contrast to the more abundant expression in myometrial tissue from pregnant women at term (Andersen 2000). Despite the lack of consensus regarding the quantitative levels of ER-β, the possibility of a role for ER-β in leiomyomata cannot be ruled out at this time, as the ER-β gene, *ESR2*, has been mapped to 14q22-24 (Enmark et al. 1997), close to the breakpoint site of one of the more common genomic rearrangements of fibroids.

Progesterone receptor-A and progesterone receptor-B. Both forms of PR (PR-A and PR-B) are expressed in leiomyomas and myometrium, with the concentration of PR-A higher than that of PR-B in both tissues (Viville et al. 1997). In one study PR-A levels were increased in leiomyomata compared with the matched myometrium (Brandon et al. 1993).

Interaction between estrogen, progesterone, and their receptors. The interaction between the two hormones and their respective receptor levels has been the subject of numerous studies and is of interest with regard to the promotion of fibroid growth. Strong evidence exists that the effect of estrogen is to increase the levels of both ER and PR in the myometrium, whereas the effect of progesterone is to decrease the level of the ER (Hsueh et al. 1975; Katzenellenbogen 1980; Thi et al. 1975). These conclusions are consistent with the sequential presentation of these two hormones during the menstrual cycle and the predominant observations that in the myometrium both ER and PR rise during the follicular (proliferative) phase and then fall during the luteal (secretory) phase of the menstrual cycle (Adams et al. 1993; Buchi and Keller 1983; Englund et al. 1998; Hsueh et al. 1975; Janne et al. 1975; Kawaguchi et al. 1991; Lessl et al. 1997; Marugo et al. 1989; Rein et al. 1990c; Sadan et al. 1987; Schmidt-Gollwitzer et al. 1979; Soules and McCarty 1982; Thi et al. 1975). Because PR levels also fall during the luteal phase, some feel that progesterone may downregulate its own receptor (Englund et al. 1998); this conclusion was also reached by Thi et al. (1975), who demonstrated a fall in PR in the myometrium of ovariectomized guinea pigs when given progesterone (Thi et al. 1975). However, the alternative explanation that the fall in PR is related to the fall in levels of estradiol during the luteal phase is difficult to exclude (Englund et al. 1998; Schmidt-Gollwitzer et al. 1979).

The majority of studies have reported the occurrence of similar cyclic rises and falls in ER and PR in uterine fibroids during the menstrual cycle, although there is some controversy regarding the degree, or the existence, of such a

fall in ER during the luteal phase. In one study, ER expression occurred throughout the menstrual cycle in leiomyomas (Kawaguchi et al. 1991). Likewise, another investigation showed that elevated levels of the ER in fibroids continue throughout the cycle, suggesting that leiomyomas may have lost a negative regulation that is maintained in the myometrium and limits the myometrial response to estrogen in the beginning of the menstrual cycle (Andersen and Barbieri 1995). On the other hand, it is clear that these tumors are subject to hormonal modulation during the cycle, as mitotic activity is reported to be significantly higher during the secretory phase than during the proliferative phase (Kawaguchi et al. 1989; Lamminen et al. 1992; Nisolle et al. 1999). These latter reports are consistent with a study by Tiltman (Tiltman 1985) that demonstrated a significantly higher mitotic activity in the leiomyomas of patients who received a progestin-only preparation. In lone contrast to these studies is an earlier report that had noted no mitotic activity in the myomas of patients given progestin therapy (Goldzieher et al. 1966). When considered in sum, however, these studies support the concept of a mitogenic effect of progesterone in fibroid tumors.

Although these data show that progesterone plays an important role in the growth of leiomyomas, it is also evident that some degree of cell proliferation occurs continuously during the menstrual cycle, as mitotic activity, albeit of a lesser degree, is present during the follicular phase of the cycle as well (Kawaguchi et al. 1989; Lamminen et al. 1992). Although the possibility of progesterone carryover effect from the luteal phase cannot be excluded, this suggests that estrogen may exert a mitogenic effect as well, and there are some clinical data (Ramcharan et al. 1981; Romieu et al. 1991) as well as tissue culture work (Chen et al. 1973; Maruo et al. 2000) to support this supposition. In addition, we might reason that the mitogenic effect of progesterone is dependent upon prior exposure to estrogen, as estrogen priming increases the concentration of PRs in myomas. In summary the evidence available suggests that during the follicular phase, estrogen upregulates ER and PR, thus setting the stage for the luteal phase progesterone surge associated with a heightened mitogenic effect and subsequent downregulation of ER and PR.

Metabolism of estradiol. The metabolism of estradiol involves a series of enzymatically catalyzed oxidative transformations, which may occur by several pathways. Because some estradiol metabolites possess significant estrogenic activity whereas others are virtually devoid of activity, the levels of the specific metabolizing enzymes and the predominant pathways employed could play important roles in fibroid tumorigenesis. Of interest, therefore, is the demonstration of alterations in two of these enzymes, 17β-hydroxysteroid dehydrogenase and estradiol 4-hydroxylase, in uterine leiomyomas.

17β-Hydroxysteroid dehydrogenase. Regardless of the phase of the cycle, the proliferative index of leiomyomas is significantly higher than that of the myometrium (Dixon et al. 2002; Kawaguchi et al. 1991; Maruo et al. 2000). This finding is not surprising in view of the elevated levels of both ERs and PRs in leiomyomas throughout the menstrual cycle. Because estradiol up-regulates both of these receptors, the increased concentration of estradiol in these tumors compared with that in the myometrium (Otubu et al. 1982) could be indicative of a pathogenetic link to the development of leiomyomata. The demonstration of reduced activity in leiomyomas of the enzyme 17β-hydroxysteroid dehydrogenase (Eiletz et al. 1980; Pollow et al. 1978b), the enzyme responsible for the conversion of estradiol to estrone, would seem to provide a plausible explanation for the accumulation of estradiol in these tumors (Otubu et al. 1982). Although estrone is weakly estrogenic, it exhibits a lower binding affinity for ERs than estradiol, and it diffuses out of the cell more rapidly than estradiol. In the myometrium, the activity of this enzyme is maximal during the early secretory phase because of upregulation by progesterone (Tseng and Gurpide 1973), resulting in a diminished estradiol effect during the second half of the cycle. In leiomyomas, on the other hand, the reduced activity of 17β-hydroxysteroid dehydrogenase may allow for the accumulation of estradiol in the cells during the secretory as well as the proliferative phase of the cycle, thus resulting in continual stimulation by estrogen, with upregulation of both the ERs and PRs, accompanied by the associated growth-promoting effects. Whether the enzymatic deficiency is a quantitative or qualitative one, and regardless of whether it is a primary or secondary development in the genesis of fibroids, the reduced activity of this enzyme could play a significant role in the pathogenesis of these tumors.

Estradiol 4-hydroxylase. Both estradiol and estrone may be metabolized by irreversible hydroxylation at several sites, including the C-2 and C-4 positions (forming catechol estrogens) and the C-6, C-15, and C-16 positions. These various hydroxylated metabolites may have quite different biologic properties. For example, the C-2 metabolites (the predominant form in humans) have limited or no activity, whereas the C-4 and C-16 metabolites possess potent estrogenicity (Martucci and Fishman 1993). For this reason, it is of great interest that the mean rate of 4-hydroxylation of estradiol is 8-fold higher than that of 2-hydroxylation in myomas, and further, that 4-hydroxylation is substantially elevated in myomas compared with surrounding myometrial tissue (Liehr et al. 1995). Because the dissociation rate of 4-hydroxyestradiol from the ER complex is also reduced compared with estradiol (Zhu and Conney 1998), this catechol metabolite may also function as a long-acting estrogen, suggesting that overexpressed 4-hydroxylase activity may play a role in the etiology of uterine fibroids (Liehr et al. 1995).

Effectors: Growth Factors and Their Receptors

The growth-promoting effects of estrogen and progesterone upon the myometrium and uterine myomas may be mediated through the mitogenic effects of growth factors produced locally by smooth muscle cells and fibroblasts (Mangrulkar et al. 1995; Rein and Nowak 1992). Growth factors are polypeptides or proteins that are secreted by a number of cell types, have a wide range of biologic effects, and generally act over short distances either in an autocrine or paracrine manner (Pusztai et al. 1993). They are essential elements in controlling the proliferation rate of cells, and overexpression of either the growth factor or its receptor may contribute to tumorigenesis. Growth factors exert most of their effects on target cells by interaction with specific cell-surface receptors, with subsequent message transmission via signal transduction systems in the cell. Even in the physiologic state, the cellular responses evoked by growth factors are complex and dependent upon a number of variables, including the cell type, the differentiation stage of the cell, other stimuli acting simultaneously upon the cell (e.g., two growth factors together may have a different effect than either one alone), and the tendency for most growth factor receptors to interact with an entire family of growth factors (Pusztai et al. 1993).

Evidence for Regulation of Growth Factors by Estrogens and Progestins

The evidence is 2-fold. First, several studies have demonstrated increases or decreases in production of particular growth factors in tissue culture cell lines or laboratory animals *in vivo* when given estrogen or progesterone (Charnock-Jones et al. 1993; Cullinan-Bove and Koos 1993; Fujimoto et al. 1997; Hyder et al. 1996; Presta 1988; Reynolds et al. 1998; Rider et al. 1997; Takahashi et al. 1994). Second, there is the indirect evidence that certain growth factors or their receptors are reduced in leiomyoma tissues from patients who are hypoestrogenic because of treatment with GnRH agonists (Lumsden et al. 1988; Rein et al. 1990b).

Although acknowledging this evidence that growth factors may be regulated by the sex steroids and simply play the role of secondary effectors in fibroid tumorigenesis, we cannot exclude the alternative possibility that

abnormal expression of a growth factor or its receptor could represent a primary event in the genesis of these tumors.

Growth Factors Identified in Fibroids

Several growth factors and their receptors have now been identified in both myometrium and leiomyomas. Those that have received the most attention in the literature include transforming growth factor (TGF)-β, bFGF, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF) (Table 3). Each will be considered briefly in summary fashion.

***Transforming growth factor*-β.** The TGF-β superfamily includes more than 30 structurally related polypeptide growth factors (Miyazono 2000), which are multifunctional cytokines that can act both as inhibitors and stimulators of cell replication (Arici and Sozen 2000). Within this large family of related factors is the TGF-β subfamily, which is composed of three major isoforms (Massague 1998) of particular interest with regard to fibroids, because they are capable not only of promoting mitogenesis but also of upregulating the synthesis of many components of the extracellular matrix, leading to fibrosis (Lyons and Moses 1990). Both of these features are characteristic of uterine fibroids. Expression of all three types of TGF-β, as well as TGF-β receptors I–III, has been detected in human myometrial tissue (Chegini et al. 1994; Tang et al. 1997). One study (Arici and Sozen 2000) found that the TGF-β3 mRNA levels in leiomyomas were 3.5-fold higher than in the myometrium, and similarly, Nowak (2000) found TGF-β3 expression to be elevated in leiomyomas compared with matched myometrium. In contrast, no significant difference was observed between fibroids and myometrium in TGF-β1 mRNA abundance (Vollenhoven et al. 1995). Although these data suggest that TGF-β3 could be important in uterine leiomyoma growth by stimulating cellular proliferation and the production of extracellular matrix, the effects of TGF-β may be either stimulatory or inhibitory, depending upon multiple factors, including the specific target cell, the concentration of TGF-β, and the presence of other growth-regulatory molecules. In low concentrations, both TGF-β1 (Battegay et al. 1990) and TGF-β3 (Arici and Sozen 2000) have elicited significant increases in smooth muscle cell proliferation, whereas at higher concentrations this effect has not been observed. Mitogenesis induced in cultures of aortic smooth muscle cells by TGF-β appears to be mediated indirectly through stimulation of autocrine secretion of PDGF, whereas higher concentrations of TGF-β result in downregulation of PDGF receptors (Battegay et al. 1990). An observed striking increase of TGF-β3 mRNA levels in luteal phase leiomyoma samples compared with those in the follicular phase suggests a pivotal role of progesterone in the regulation of TGF-β3 expression (Arici and Sozen 2000). In contrast, no variation was observed in one study in the expression of TGFβ mRNAs and proteins in myometrial tissue during the menstrual cycle (Chegini et al. 1994), and other investigators concluded that TGF-βs had no significant effect on myometrial cell proliferation (Tang et al. 1997).

In view of the probable role of this growth factor in fibroid pathophysiology, it is of particular interest that the gene coding for TGF-β3 is located near the 14q23-24 breakpoints (Andersen 1998), one of the most common translocation sites identified in cytogenetic studies of fibroids.

***Basic fibroblast growth factor*.** bFGF causes proliferation of smooth muscle cells, including leiomyoma and myometrial cells (Stewart and Nowak 1996), and also promotes angiogenesis. This factor can also bind to a component of the extracellular matrix (Dixon et al. 2000; Mangrulkar et al. 1995). In one study there was much stronger immunohistochemical staining for bFGF in fibroids than in the myometrium because of the large amount of extracellular matrix in uterine myomata; this finding led the authors to conclude that large quantities of bFGF are stored in the extracellular matrix of these tumors (Mangrulkar et al. 1995). In addition, increased expression of bFGF mRNA was found in the leiomyomas compared with the myometrium. Some immunoreactivity for the FGF type 1 receptor in the extracellular matrix of leiomyomas has been demonstrated, although the cellular staining for the receptor was greater in the myometrium than in the leiomyomas (Anania et al. 1997).

Thus, apparently both TGF-β3 and bFGF are overexpressed in leiomyomas compared with matched myometrium, and both factors may contribute to the enhanced growth of leiomyomas. Indeed, Stewart and Nowak feel that these two factors may be central to the pathogenesis of uterine leiomyomas (Stewart and Nowak 1998).

***Epidermal growth factor*.** EGF is mitogenic for the cells of both myometrium and leiomyomas in tissue cultures (Fayed et al. 1989). Equally important, and possibly a unique feature of this factor, is its apparent upregulation in fibroids by progesterone (Maruo et al. 2000). The concentration of EGF mRNA in leiomyomas is similar to that of the myometrium during the follicular phase but significantly elevated in leiomyomas during the luteal phase, whereas the concentration in the myometrium remains essentially unchanged (Harrison-Woolrych et al. 1994). Because the mitotic activity of leiomyomas is maximal during the luteal phase of the cycle, this finding suggests that the production of EGF may be one mechanism through which progesterone stimulates mitotic activity in fibroids.

The mRNA for the EGF receptor has been detected in both myometrial and leiomyoma cells (Yeh et al. 1991). Although the levels of EGF receptors are not significantly higher in leiomyomas than in the myometrium and do not seem to fluctuate during the menstrual cycle (Chegini et al. 1986; Hofmann et al. 1984; Lumsden et al. 1988), there is a sharp reduction of EGF-receptor levels in the leiomyomas but not in the myometrium of women treated with GnRH agonists prior to surgery (Lumsden et al. 1988). These data suggest that the EGF receptors in fibroids are more sensitive to regulation by the ovarian sex steroids than those in the myometrium. More importantly, because the reduction of EGF receptor levels correlates with shrinkage of the fibroids as a result of the GnRH-agonist

Table 3. Potential effectors and their receptors implicated in leiomyoma pathobiology.[a]

Factor/receptor	Elevated?	Luteal?	Mitogenic?	Reference
TGF-β_3	Yes	Yes	Yes, low concentration	Arici and Sozen 2000
TGF-β_3 receptor	?	?	–	
bFGF	Yes	?	Yes	Mangrulkar et al. 1995
bFGF receptor	No	?	–	
EGF	Yes	Yes	Yes	Harrison-Woolrych et al. 1994
EGF receptor	No	No	–	
PDGF	No	?	Yes, in conjunction with EGF or IGF	Fayed et al. 1989
PDGF receptor	Yes	?	–	
VEGF	No	No	No	Harrison-Woolrych et al. 1995
VEGF receptor	?	?	–	
IGF-I	Yes	(Late follicular)	Yes	Boehm et al. 1990
IGF-I receptor	Yes	No	–	
IGF-II	Yes	No	No	Vollenhoven et al. 1993
IGF-II receptor	No	No	–	
Prolactin	Yes	?	?	Nowak et al. 1999
Prolactin receptor	?	?	–	

[a]Lists whether the factor is elevated in leiomyomas compared with myometrium, elevated during the luteal phase, and/or associated with mitogenic activity.

therapy, it suggests that the effects of sex steroids on fibroid growth may be mediated, in part, by EGF (Rein and Nowak 1992). In this regard, it is of interest that in cultures of leiomyoma cells, estradiol augmented the expression of the EGF receptor, whereas progesterone increased the expression of EGF, suggesting to the authors that estradiol and progesterone may act in combination to stimulate proliferation in fibroids through the induction of EGF and its receptor (Maruo et al. 2000).

Platelet-derived growth factor. PDGF is a potent mitogen for vascular smooth muscle cells and another of the heparin-binding growth factors along with bFGF and VEGF. Because of the capacity of these factors to bind to heparin, they may become sequestered in the extracellular matrix, which is typically abundant in fibroids and may therefore serve as a reservoir for these growth factors (Nowak 1999). The mRNA for PDGF is expressed in leiomyomas, but the levels are similar to those found in the myometrium (Boehm et al. 1990). On the other hand, significantly more PDGF receptor sites per cell are seen in leiomyomas than in the myometrium, although the PDGF receptor binding affinity in the tumor cells is lower than that of the myometrium (Fayed et al. 1989).

Perhaps the most interesting aspect of PDGF in leiomyomas, however, may not be its growth factor role, acting in isolation, but rather its action in conjunction with other growth factors such as EGF and IGFs. For example, when myometrial cells are treated with both PDGF and EGF, there is a synergistic decrease in DNA synthesis, whereas treatment of leiomyoma cells with both factors results in an additive increase in DNA synthesis (Fayed et al. 1989). Insulin and PDGF exert an additive effect upon DNA synthesis in myometrial and leiomyoma cells (Fayed et al. 1989); previous studies using other cell systems have found that target cells must have prior exposure to a competence growth factor such as PDGF before IGF stimulation will promote movement through the cell cycle (Pledger et al. 1978; Stiles et al. 1979).

Vascular endothelial growth factor. Five VEGF isoforms have been identified (Neufeld et al. 1999). All but one (VEGF-121) contain heparin-binding regions that can mediate binding to the extracellular matrix (Hyder et al. 2000), which may thus serve as a reservoir for this factor as with the other heparin-binding factors bFGF and PDGF. Although VEGF seems to be a highly specific mitogen for vascular endothelial cells, VEGF mRNA and VEGF protein expression have now been identified in the smooth muscle cells of both myometrium and leiomyomata (Dixon et al. 2000; Harrison-Woolrych et al. 1995), and VEGF receptors have been demonstrated in the smooth muscle cells of the myometrium (Brown et al. 1997). Leiomyomata apparently do not have significantly different levels of VEGF mRNA than the myometrium, do not exhibit differences in VEGF mRNA levels between the proliferative and secretory phases of the cycle, and show similar levels of VEGF mRNA after treatment with a GnRH analog (Harrison-Woolrych et al. 1995).

Despite these findings, and evidence that VEGF is not mitogenic to smooth muscle cells (Ferrara et al. 1992), interest remains in the potential role of this factor in fibroid growth, for several reasons. VEGF stimulates angiogenesis, which is essential for actively growing tumors, and VEGF is the most potent agent known for increasing capillary permeability, which could enhance the growth of fibroids by increasing their nutrient supply. VEGF could also have an indirect effect by inducing the proliferation of endothelial cells, which themselves produce a number of growth factors. In addition, VEGF acts synergistically with fibroblast growth factor (FGF) (Hyder et al. 2000), and it can release the angiogenic factor bFGF from its storage on heparan sulfates of the extracellular matrix (Jonca et al. 1997), with the resulting combination of the two angiogenic mitogens having a synergistic effect on angiogenesis (Asahara et al. 1995; Goto et al. 1993). Further, the resulting availability of bFGF permits the expression of its mitogenic effect upon the smooth muscle cells.

Insulin-like growth factor. The IGFs have received considerable attention in the literature. The family of IGFs consists of two IGFs (IGF-I and IGF-II), two cell membrane receptors (IGF-IR and IGF-IIR), and six IGF binding proteins (Yu and Berkel 1999). Thus, the actions of the IGFs are mediated through the IGF receptors, primarily IGF-IR, and are regulated by the IGF-binding proteins. The IGFs are produced by most tissues of the body, are abundant in the circulation, and have the potential to act through endocrine, autocrine, and paracrine mechanisms (Cohick and Clemmons 1993). These factors are structurally related to proinsulin and promote cellular proliferation, differentiation, and cell survival (Strawn et al. 1995; Yu and Berkel 1999). Evidence exists for dissimilar roles of the two IGFs, in that IGF-II appears to be primarily responsible for the terminal differentiation of skeletal muscle cells and the down-regulation of IGF-I receptor gene expression, whereas IGF-I is responsible for myogenesis (Rosenthal et al. 1994; Strawn et al. 1995). In most situations the IGF binding proteins inhibit the actions of IGFs by blocking their binding to the receptor; in certain circumstances, however, these binding proteins may be able to enhance the action of IGF-I by binding to it and preventing its degradation, thereby increasing its bioavailability in target tissues (Yu and Berkel 1999).

Several investigators have identified mRNAs for IGF-I and IGF-II and their receptors in both the myometrium and fibroid tumors. IGF-I, but not IGF-II, was mitogenic in leiomyoma cell cultures (Strawn et al. 1995). The levels of IGF-I mRNA were reported higher in leiomyomas than in the myometrium in two studies (Boehm et al. 1990; Hoppener et al. 1988), whereas two other studies concluded that the levels were not significantly different (Gloudemans et al. 1990; Vollenhoven et al. 1993). Increased IGF-I peptide has been detected in some, but not all, leiomyomata compared with myometrium in immunohistochemical studies (Dixon et al. 2000). The variation in relative amounts of IGF-I mRNA reported in these studies may have been due to the heterogeneity that exists among fibroid tumors (Rein and Nowak 1992). In three of these studies (Boehm et al. 1990; Hoppener et al. 1988; Vollenhoven et al. 1993) the mRNA levels of IGF-II were higher in leiomyomas than in the myometrium, whereas one study reported low levels in both tissues (Gloudemans et al. 1990). Giudice et al. (1993) found the IGF-I gene expression to be most abundant in leiomyomata during the late proliferative phase of the cycle, suggesting that estrogen upregulates this growth factor in leiomyomas; on the other hand, IGF-II gene expression did not vary with the phase of the cycle.

Both IGFs can bind to the IGF-I receptor with similar affinity, whereas the IGF-II receptor preferentially binds IGF-II (Van der Ven et al. 1997). The IGF-I receptor mediates most of the biologic actions of both IGF-I and IGF-II (Cohick and Clemmons 1993), including the mitogenic, metabolic, and cell-survival properties of IGFs through tyrosine kinase signaling activity. The IGF-II/mannose 6-phosphate receptor appears to be a bifunctional receptor serving as both a lysosomal enzyme-targeting system and a suppressor of the action of IGF-II by increasing its degradation (Nissley and Lopaczynski 1991; Oates et al. 1998). The levels of IGF-I receptors in leiomyomas have been reported to exceed those of the myometrium in three studies (Chandrasekhar et al. 1992; Tommola et al. 1989; Van der Ven et al. 1997), whereas Chandrasekhar et al. found no difference in the levels of the IGF-II receptors. The levels of neither IGF-I nor IGF-II receptors seem to vary with the stage of the menstrual cycle (Giudice et al. 1993).

The conclusion of most of these studies has been that IGF-I may play a mitogenic role in the growth of uterine fibroids because of increased levels of IGF-I receptors and overexpression of the growth factor itself. Lower levels of the IGF binding protein-3 in leiomyomas than in myometrium could also be significant, as this would increase the bioavailability of free

bioactive IGF in fibroids (Vollenhoven et al. 1993).

Prolactin. Although initially identified as a pituitary gland hormone, several studies have demonstrated that prolactin is also produced by uterine tissues, including the endometrium, myometrium, and uterine leiomyomas (Daly et al. 1984; Maslar and Riddick 1979; Walters et al. 1983). The significance of prolactin production in leiomyomas is not yet well defined; however, interest in this hormone has been stimulated by the finding that prolactin acts as a mitogen for vascular smooth muscle (Sauro and Zorn 1991). In addition, in one study of myometrial and leiomyoma explant cultures, fibroid prolactin secretion was substantially greater than myometrial prolactin secretion (Rein et al. 1990a). On the other hand, Daly et al. found that estrogen enhanced the secretion of prolactin in fibroid tissue cultures, whereas progesterone exhibited a suppressive effect (Daly et al. 1984). Because leiomyomas are mitotically active during the luteal phase, the inhibition of leiomyoma prolactin production by progesterone tends to cast some doubt upon the role of this hormone in fibroid growth. However, in a recent study, treatment of leiomyoma and myometrial cell cultures with a prolactin-neutralizing antibody inhibited cell proliferation, leading the authors to conclude that prolactin may be an autocrine or paracrine growth factor for both leiomyoma and myometrial cells (Nowak et al. 1999). At this date, it would seem that the prolactin story is unfinished, evolving, and worthy of further study.

Summary of Growth Factors

From this brief review of the major growth factors identified in fibroids thus far, we can surmise that multiple growth factors are probably important in the pathogenesis of these tumors. Different growth factors could play a role at different stages of the disease (Newbold et al. 2000). Many of the factors may interact, sometimes resulting in a synergistic effect, as demonstrated by the two angiogenic mitogens VEGF and bFGF. In other situations, the effect of one growth factor is dependent upon the presence of another, exemplified by IGF-I acting as a progression factor in the cell cycle when competence factors such as PDGF and FGF are also present (Cohick and Clemmons 1993), and by the indirect mitogenic effect of TGF-β resulting from the stimulation of PDGF secretion (Battegay et al. 1990).

Conclusions

In this overview of the etiology and pathogenesis of uterine fibroids, we have attempted to analyze the literature and present prevailing evidence and opinions. Although research in this area has been lacking in the past, much has been learned about this extremely common public health problem during the last 20 years (McBride 1999; Newbold et al. 2000). We briefly summarize some of these data in the following conclusions:

- Risk factors for fibroids may achieve significance through their contribution to either the initiation or promotion phases of tumorigenesis. Although their impact often appears related to their effect upon estrogen and progesterone, other mechanisms may be involved. For example, early menarche increases the overall estrogen exposure, but also involves more menstruations with their concomitant tissue damage. Two of the more consistent risk factors that have been identified are age (late reproductive years) and African-American ethnicity. The effect of age may reflect more opportunity for dysregulated cells to be produced or, alternatively, a prolonged period for growth under the hormonal influences of the reproductive years. Why African-American women are at higher risk for clinically significant fibroids is not known, but apparent metabolic differences could increase the estrogenic promotional effect, such as the predilection for the 16α hydroxylation of estradiol metabolic pathway. Increased risk has also been associated with early menarche, nulliparity, and obesity, whereas decreased risk has been found with increasing parity and smoking. On the basis of clinical reports, tamoxifen also appears to be a risk factor.
- Karyotypic abnormalities have been identified in approximately 40% of surgically removed uterine leiomyomas. The most common of these are the translocation t(12;14) and the deletion of 7q; however, these abnormalities do not exclude submicroscopic mutations of a more universal nature, which will require molecular demonstration. There may be more than one genetic pathway to the formation of fibroids. Phenotypic fibroid variants are probably related to chromosomal differences, either from the outset or as a result of clonal evolution.
- Estrogen and progesterone appear to be promoters of fibroid growth, acting in concert. Thus, estrogen upregulates both ERs and PRs during the follicular phase, followed by progesterone-induced mitogenesis during the luteal phase. The deficiency of the estrogen-metabolizing enzyme 17β-hydroxysteroid dehydrogenase in fibroids may be responsible for the accumulation of estradiol in these tumors and its consequent growth-promoting effects. Likewise, the overexpression of estradiol 4-hydroxylase seems highly significant, as the resulting metabolite possesses long-acting estrogenic activity.
- The levels of several growth factors and their receptors are increased in fibroids. TGF-β3 and bFGF may be especially important in the pathogenesis of these tumors in view of their combined mitogenic effect and promotion of extracellular matrix production. EGF appears to be significant, as it is the only characterized growth factor, other than TGF-β3, with elevated expression during the luteal phase, when leiomyoma mitotic activity is maximal. IGF-I almost certainly plays an important role because of its potent mitogenic capacity and the overexpression of both the peptide and its receptor in leiomyomas. Growth factors may be the mediators or effectors of sex steroid upregulation, but a primary dysregulation of one or more growth factors must also be considered.
- Finally, the most important piece of the fibroid puzzle, the initiator(s), remains unsolved. Further elucidation of the genetic and molecular changes will provide insights into the pathobiology of these tumors and may offer clues to initiating conditions responsible for such changes. Considering the extremely high incidence of fibroids, evidently within all races and all geographic areas of the world, we believe that the initiating conditions must be common to most or all women. Because the presence of fibroids offers no known advantages to affected women, but rather considerable morbidity in many cases, one is challenged to fathom the evolutionary basis for the development of an organ so prone to tumor formation, albeit benign tumors. Perhaps there are conditions existing today that significantly impact uterine physiology that were not prevalent in antiquity. There can be little doubt that women in the past experienced fewer menstrual periods because of their shorter life spans, more demanding physical conditions, and prolonged breastfeeding. Under such circumstances the presumed reproductive advantages offered by a hemochorial placenta (Campbell and Cameron 1998) and the occasionally menstruating uterus might have been enjoyed, with only the exceptional disadvantage of rarely developing uterine leiomyomas. Other changes in modern lifestyle, such as dietary shifts to a higher-fat, lower-fiber diet and the potential impact of environmental estrogens, could also be significant because of increased estrogen exposure.

On the basis of our current state of knowledge, we can only speculate upon the initiators of this common condition. Future research efforts may provide a better understanding, however, of the causes and mechanisms of uterine fibroid tumorigenesis. Insights resulting from elucidation of the basic biology of these tumors might then be successfully translated into preventative strategies that will reduce the incidence and/or morbidity of this disease.

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