Sex steroidal regulation of uterine leiomyoma growth and apoptosis

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Uterine leiomyomas develop during the reproductive years and regress after menopause, indicating ovarian steroidal-dependent growth potential. Although the clinical and biochemical observations have traditionally supported an important role for estrogen in the promotion of leiomyoma growth, there is also increasing evidence to suggest the involvement of progesterone in the pathogenesis of leiomyoma. In this review, much attention has been paid to characterizing the molecular mechanisms of sex steroidal regulation of leiomyoma growth and apoptosis by evaluating the effects of sex steroids on the expression of growth factors and apoptosis-related factors. The effects of GnRH agonist on the expression of these factors in leiomyoma are also described. 17ß-Estradiol up-regulates epidermal growth factor (EGF) receptor, but down-regulates p53 protein in leiomyoma cells, whereas progesterone augments EGF and Bcl-2 protein, but inhibits insulin-like growth factor (IGF-I) and tumour necrosis factor (TNFα). Since it is now evident that EGF and IGF-I act as local factors which stimulate leiomyoma growth, these findings suggest that progesterone may have dual actions, stimulatory and inhibitory, on leiomyoma cell growth and survival, depending on the local growth factor conditions around each leiomyoma. This may explain why the size of uterine leiomyomas during the use of levonorgestrel-releasing intrauterine system (LNG-IUS) increases in some but decreases in other instances. This may also explain why the size of leiomyomas during pregnancy does not increase despite the overwhelming increase in circulating concentrations of sex steroid hormones. Moreover, there is further evidence to suggest that the interactions between estrogen receptors and progesterone receptors may be involved in the modulation of gene transcription activity in leiomyoma. This review demonstrates that leiomyoma growth is integrally regulated by the complex cross-talk between sex steroid hormones and growth factors.

Key words: apoptosis/GnRH agonist/growth factor/leiomyoma/sex steroid hormone

Introduction

Uterine leiomyoma is the most common benign smooth muscle cell tumour of the myometrium, occurring in as many as 30% of women aged >35 years (Vollenhoven et al., 1990). Evaluation of a glucose-6-phosphate dehydrogenase marker in leiomyoma suggests that it is a proliferation of a single clone of smooth muscle cells (Fletcher et al., 1990). Cytogenetic studies provide further evidence of the clonal nature of the smooth muscle cell proliferation in leiomyoma (Pandis et al., 1991; Meloni et al., 1992). Although the nature of the initial event is unknown, ovarian steroids play a central role in leiomyoma growth because leiomyomas grow during the reproductive years and regress after menopause (Rein and Nowak, 1992). Furthermore, treatment with GnRH agonists, which reduce ovarian hormone production, lead to a reduction in the size of leiomyomas, but re-enlargement of leiomyomas occurs after therapy with GnRH agonist is discontinued (West et al., 1987). These findings suggest that leiomyoma growth is dependent on ovarian sex steroids.

Homeostatic control of the net growth of tumours is the result of the dynamic balance between cell proliferation and cell death (Wyllie et al., 1980). However, the molecular mechanisms underlying the action of ovarian sex steroids in the regulation of the proliferation and apoptosis of leiomyoma cells remain to be elucidated. Thus, in this review much attention has been paid to evaluate the effects of sex steroids on the expression of local growth factors and apoptosis-related factors in leiomyoma cells. There is increasing evidence to suggest that sex steroids are not the sole modulators of leiomyoma tumorigenesis and growth. Steroid hormone levels in women with leiomyomas are similar to those in normal women (Buttram, 1986). In addition, leiomyomas are not observed frequently in conditions such as polycystic ovarian syndrome in which there is chronic estrogen elevation. Finally, the heterogeneity of leiomyoma growth within the same uterus, despite the identical exposure to circulating sex steroid concentrations, suggests the involvement of local growth factors. Growth factors have been reported to be differently expressed...
between leiomyoma and myometrium, and mediate the effects of sex steroid hormones. This review provides current knowledge on the interrelations among sex steroids, growth hormones, and apoptosis-related factors in regulating the proliferation and apoptosis of leiomyoma cells.

Sex steroid hormones and their receptors in uterine leiomyomas

Estrogen

The accumulating evidence supports the concept that estrogen is closely related to the tumorigenesis and growth of leiomyoma. Estrogen exerts its physiological effects on the target cells by binding to specific nuclear receptors, of which two subtypes are known: the estrogen receptor α (ERα) and ERβ. ERβ has considerable homology to ERα in the DNA-binding domain and the ligand-binding domain (Mosselman et al., 1996).

Several studies have demonstrated that both ERα and ERβ mRNA are expressed in myometrium and leiomyoma (Pedeutour et al., 1998; Benassayag et al., 1999; Kovács et al., 2001; Sakaguchi et al., 2003). Sakaguchi et al. (2003) have reported that both ERα and ERβ mRNA levels in the myometrium change in a similar manner during the menstrual cycle, but that ERα mRNA levels predominate over ERβ mRNA levels. Two authors have reported that ERα and ERβ mRNA levels are elevated in leiomyoma compared with myometrium (Benassayag et al., 1999; Kovács et al., 2001). It has been reported that both ERα and ERβ can stimulate transcription of the target genes in a similar manner, although the degree of activation of ERβ is lower than that of ERα (Enmark and Gustafsson, 1999). Several estrogen-regulated genes have been shown to have elevated expression in leiomyomas compared with autologous myometrium, including connexin 43 gap junction protein, type I and III collagen, IGF-I and its receptor, parathyroid hormone-related peptide, and progesterone receptor (PR) (Andersen et al., 1995).

Moreover, several authors have demonstrated that leiomyomas over-express aromatase p450, an estrogen synthetase, which catalyses androgens to estrogens, and that in situ estrogen synthesized in leiomyoma may play a role in the promotion of leiomyoma growth in an autocrine/paracrine mechanism (Bulun et al., 1994; Shozu et al., 2001, 2002). Shozu et al. (2001) have reported that GnRH agonist therapy inhibits the expression of aromatase P450 in leiomyoma cells, suggesting that the suppression of in situ estrogen can be an additional mechanism of GnRH agonist-induced regression of leiomyoma.

A recent report has demonstrated that estrogen mediates the mitogenic effects on leiomyoma cells by triggering the rapid and transient activation of the mitogen-activated protein kinase pathway and that the early downstream signal transduction events determined by 17β-estradiol (E2) stimulation, including the rapid protein tyrosine phosphorylation of intracellular proteins such as growth-associated protein (GAP), phosphatidylinositol 3-kinase (PI-3-K), and phospholipase C (PLCγ) and the activation of ancillary protein kinases, are related to E2-induced platelet-derived growth factor (PDGF) secretion (Barbarisi et al., 2001). Furthermore, accumulating evidence suggests that the action of estrogen may be mediated in part by growth factors such as epidermal growth factor (EGF), insulin-like growth factor (IGF-I) and PDGF produced by the target cells in the uterus (Huet-Hudson et al., 1990; Murphy and Ghahary, 1990; Nelson et al., 1992; Barbarisi et al., 2001).

Progesterone

Several lines of the clinical and biochemical evidence have implicated a critical role for progesterone in the pathogenesis of leiomyoma (Maruo et al., 2000; Rein, 2000). It has been suggested that progesterone may stimulate the mitotic activity and proliferation of leiomyoma. Kawaguchi et al. (1989) found the increased mitotic activity in leiomyoma at the secretory phase of the cycle, suggesting that leiomyoma growth is affected by progesterone levels. Tiltman (1985) reported that administration of medroxyprogesterone acetate significantly increased the mitotic activity in leiomyoma compared with the untreated control group. Treatment with a progesterone antagonist RU-486 (mifepristone) has been reported to induce the regression of leiomyoma (Murphy et al., 1993, 1995; Kettel et al., 1994) with a reduction in PR immunoreactivity (Murphy et al., 1993), suggesting a direct antiestrogenic effect. Conversely, progestin can inhibit GnRH agonist-induced shrinkage of leiomyoma (Friedman et al., 1988; Cart et al., 1993). Brandon et al. (1993) demonstrated increased PR mRNA and protein levels in leiomyoma together with elevated proliferation-associated antigen Ki-67 in comparison to adjacent myometrium, suggesting the association of amplified progesterone-mediated signalling with leiomyoma growth. These findings support the view that progesterone may play a vital role in promoting leiomyoma growth.

PR exists in two distinct forms, termed PR-A and PR-B (Kastner et al., 1990). These receptor functions as ligand-activated transcription factors, but both receptor isoforms exhibit distinct biological functions. PR-B functions as a transcriptional activator of progesterone-responsive genes (Wen et al., 1994), whereas PR-A acts as a potent ligand-dependent repressor of PR-B transcriptional activity in promoter and cell contexts where PR-A is inactive as a transcriptional activator (Vegeto et al., 1993). There is the complex cross-talk between ER and PR signalling pathways, as shown by the observations that estrogen can induce PR expression in myometrial cells of the rhesus monkey (Okulicz et al., 1989) and transformed hamster myocytes (Sadovsky et al., 1993), and increase the transcription rate of the PR-B gene in human breast cancer cells (Graham et al., 1995), whereas both PR isoforms can act as potent ligand-dependent repressors of ER activity (Kraus et al., 1995). Moreover, progesterone down-regulates E2-stimulated PR transcription (Graham et al., 1995; Hodges et al., 2002).

Both PR-A and PR-B have been identified in leiomyoma and myometrium. Two investigators have demonstrated that PR-A and PR-B contents are higher in leiomyoma than in adjacent myometrium with a significant dominance of PR-A over PR-B (Viville et al., 1997; Nisolle et al., 1999). However, Viville et al. (1997) failed to find the difference between the concentrations of mRNA encoding PR-A and PR-B in leiomyoma and myometrium, suggesting the post-translational control. In addition, GnRH agonist down-regulates immunoreactive PR, PR-A and PR-B expression, and PR mRNA levels in leiomyoma (Vu et al., 1998; Nisolle et al., 1999; Wu et al., 2002a). Interestingly, Fujimoto et al. (1998) have found the relative over-expression of PR-B mRNA in...
the surface of leiomyoma, suggesting that the predominant expression of PR-B in this part reveals an activated phenotype for progestational proliferation related to the growth of leiomyoma. However, it remains unknown whether high levels of PR-A are associated with the reduced progesterone responsiveness of leiomyoma cells.

Sex steroidal regulation of growth factor expression

Epidermal growth factor and its receptor

Epidermal growth factor (EGF) has been demonstrated to play a crucial role as a local growth factor in regulating leiomyoma growth (Hofmann et al., 1984; Huet-Hudson et al., 1990; Nelson et al., 1991; Yeh et al., 1991; Rossi et al., 1992). Leiomyoma and myometrium contain specific, high affinity binding sites for EGF (Hofmann et al., 1984). The expression of EGF mRNA and EGF receptor (EGF-R) mRNA in myometrial and leiomyoma cells suggest that EGF may be involved in the autocrine/paracrine regulation of the growth of these tissues (Yeh et al., 1991).

The effect of E2 may be mediated by EGF in murine uterine tissues and EGF is capable of replacing E2 in the stimulation of female genital tract growth (Nelson et al., 1991). Actually, treatment with either E2 or EGF significantly increases not only the autoradiographic uptake of [3H]thymidine by cultured leiomyoma cells but also the percentage of proliferating cell nuclear antigen (PCNA)-positive nuclei of those cells relative to those in untreated cultures (Maruo et al., 1996). However, the stimulatory effects of the combined treatment with E2 and EGF on the PCNA-positive rate are not additive, suggesting that E2 and EGF may act in the same channel to stimulate the proliferative activity of leiomyoma cells (Maruo et al., 1996).

The presence of immunoreactive EGF protein and EGF mRNA has been reported in myometrial cells (Rossi et al., 1992; Yeh et al., 1991). Leiomyoma has significantly higher amounts of EGF mRNA than myometrium in the secretory phase of the menstrual cycle (Harrison-Woolrych et al., 1994). A possible involvement of sex steroid hormones in the regulation of EGF has been postulated because GnRH agonist treatment causes a significant reduction not only in the specific binding of EGF to leiomyoma cells but also in EGF mRNA in leiomyoma cells compared with the untreated group (Lumsden et al., 1988; Harrison-Woolrych et al., 1994). The addition of either E2 or progesterone increases PCNA expression in cultured leiomyoma cells compared to that in control cultures (Maruo et al., 1996). The fact that progesterone up-regulates PCNA protein expression in leiomyoma cells is in good agreement with the in vivo finding of a higher PCNA labelling index in leiomyoma in the secretory phase of the cycle compared to that in the proliferative phase. Furthermore, the PCNA labelling index in leiomyoma is significantly higher than that in the adjacent myometrium throughout the menstrual cycle. This may permit the enhanced growth of leiomyoma over the adjacent myometrium in the same uterus (Shimomura et al., 1998).

Leiomyoma cells contain EGF protein with a molecular mass of 133 kDa (Shimomura et al., 1998). The addition of progesterone remarkably increases 133 kDa immunoreactive EGF expression together with the appearance of 71 kDa immunoreactive EGF in leiomyoma cells compared to that in control cultures (Figure 1) (Shimomura et al., 1998). EGF is a 6 kDa polypeptide that is generated by proteolytic processing of a larger molecular precursor, 133 kDa prepro-EGF (Gray et al., 1983; Scott et al., 1983). EGF is shown to be present in its prepro-form in the kidney and other tissues (Salido et al., 1990). Taking these findings into account, the immunoreactive EGF proteins with higher molecular masses of 133 kDa and 71 kDa induced by progesterone treatment in cultured leiomyoma cells are postulated to be a prepro-EGF-like protein and an active species generated from the prepro-EGF protein respectively. In contrast, the addition of E2 results in a somewhat lower expression of 133-kDa immunoreactive EGF (Figure 1) and in the augmentation of EGF-R expression in leiomyoma cells compared to that in untreated cultures, but P4 does not (Shimomura et al., 1998). These results indicate that progesterone up-regulates the expression of PCNA and immunoreactive EGF in leiomyoma cells, whereas E2 up-regulates the expression of PCNA and EGF-R in those cells. The progesterone-induced increase in PCNA expression in leiomyoma cells may be mediated by the progesterone-induced EGF-like proteins in the cells, whereas the E2-induced increase in PCNA expression in leiomyoma cells may be mediated by the E2-induced EGF-R in those cells. It is therefore conceivable that progesterone and E2 act in combination to stimulate the proliferative potential of leiomyoma cells through the induction of EGF-like proteins and EGF-R expression in leiomyoma (Shimomura et al., 1998).

Insulin-like growth factor (IGF)-I, IGF-II and their receptors

Insulin-like growth factor (IGF)-I and IGF-II are polypeptide growth factors structurally related to proinsulin (Duan, 2002). IGF-I is a major anabolic agent responsible for growth and differentiation, and mediates the biological effects of growth hormone (GH) in many cell types. The biological actions of IGF are mediated by the IGF-I and IGF-II receptors (Duan, 2002). IGF-I receptors, but not IGF-II receptors, are increased in leiomyoma compared with those in myometrium (Chandrasekhar et al., 1992). IGF-I and IGF-II receptor mRNA are not dependent on the menstrual cycle stages (Giudice et al., 1993). Earlier studies

Sex steroids and leiomyoma

Figure 1. Effects of sex steroids on EGF-like protein expression in cultured leiomyoma cells, as assessed by Western immunoblot analysis. The addition of progesterone (100 ng/ml) resulted in a remarkable increase in 133 kDa immunoreactive EGF expression together with the appearance of 71 kDa immunoreactive EGF, whereas the addition of 17β-estradiol (E2) (10 ng/ml) resulted in a somewhat lower expression of 133 kDa immunoreactive EGF relative to that in control cultures. Adapted from Shimomura et al. (1998) by permission of The Endocrine Society. © Copyright 1997, 1998, 2001 and 2002. The Endocrine Society.
demonstrated the presence of IGF-I and IGF-II mRNA in leiomyoma (Höppener et al., 1988; Boehm et al., 1990; Gloudemans et al., 1990) and myometrium (Boehm et al., 1990; Gloudemans et al., 1990). Several authors have reported elevated levels of IGF-I peptide (Van der Ven et al., 1994, 1997), IGF-I mRNA (Boehm et al., 1990; Giudice et al., 1993; Englund et al., 2000), IGF-II mRNA (Boehm et al., 1990; Vollenhoven et al., 1993) and IGF-I receptor (Tommola et al., 1989; Chandrasekhar et al., 1992; Van der Ven et al., 1997; Dixon et al., 2000) in leiomyoma compared with myometrium. These findings suggest that IGF may act to promote leiomyoma growth in an autocrine/paracrine fashion. In fact, IGF-I has been shown to act as a mitogen in leiomyoma cells. IGF-I significantly increases cell number (Strawn et al., 1995) and proliferation rate in cultured leiomyoma cells (Van der Ven et al., 1994). Gao et al. (2001) have demonstrated that IGF-I significantly increases the expression of immunoreactive PCNA and augments cell proliferation in cultured leiomyoma cells compared with those in untreated cultures.

IGF-I acts as a survival factor to inhibit apoptosis in a variety of cell types (Jung et al., 1996; Matthews and Feldman, 1996; Parrizas and Leroith, 1997; Wang et al., 1998). Accordingly, the over-expression of IGF-I receptor in the cells increases the tumorigenic potential of the cells and protects the cells from apoptosis (Resnicoff et al., 1995; Parrizas et al., 1997). Gao et al. (2001) have demonstrated that IGF-I significantly increases Bcl-2 protein in cultured leiomyoma cells and augments cell survival of these cells compared with those in untreated cultures. Furthermore, IGF-I significantly decreases the apoptosis of leiomyoma cells compared with that of untreated cultures (Gao et al., 2001). These results suggest that IGF-I plays crucial roles in leiomyoma cell growth not only in promoting the proliferative potential through up-regulation of PCNA expression but also in inhibiting apoptosis through up-regulation of Bcl-2 protein expression.

IGF-I has been demonstrated to mediate estrogen action in the animal uterus. IGF-I regulates the growth-promoting effects of sex steroids in rhesus monkey uterus (Adesanya et al., 1996). Administration of E2 increases IGF-I mRNA in the ovariectomized rat uterus (Norstedt et al., 1989) and IGF-I receptor in the immature rat uterus (Ghahary and Murphy, 1989). Several studies have demonstrated that IGF-I induced by estrogen in the uterus can replace estrogen not only in mediating the mitogenesis (Murphy et al., 1987; Murphy and Ghahary, 1990; Pollard, 1990) but also in inducing PR (Katzenellenbogen and Norman, 1990). In humans, Giudice et al. (1993) reported that IGF-I mRNA in leiomyoma was most abundant during the late proliferative phase of the menstrual cycle, but IGF-II mRNA expression was not dependent on the cycle. Sex steroid hormones affect IGF-I mRNA levels, but not IGF-I receptor mRNA levels, in cultured leiomyoma cells. Treatment with progesterone alone or combined treatment with E2 and progesterone significantly decreases IGF-I mRNA expression in cultured leiomyoma cells compared with those in untreated cultures, whereas treatment with E2 alone does not affect IGF-I mRNA expression in those cells (Figure 2) (Yamada et al., 2004). No significant differences are noted in IGF-I receptor mRNA expression between untreated cultures and cultures treated with either E2 or progesterone (Yamada et al., 2004). These results provide the evidence that progesterone down-regulates IGF-I expression in cultured leiomyoma cells without affecting IGF-I receptor expression in those cells. In addition, the explant cultures of leiomyoma and myometrium from women treated with GnRH agonist secrete significantly less IGF-I and IGF-II compared with untreated tissues (Rein et al., 1990). GnRH agonist administration is associated with a decrease in both IGF-I mRNA levels (Englund et al., 2000) and immunoreactive IGF-I receptor expression (Di Lieto et al., 2003). These results suggest that IGF-I may be involved in the regulation of leiomyoma growth as a local mediator of the growth-promoting action of sex steroids.

Six IGF-binding proteins (IGFBP) have been characterized (Drop et al., 1992). IGFBP bind IGF-I and IGF-II with equal affinity and modulate IGF binding to receptors, resulting in the inhibition of IGF actions by preventing them from gaining access to the receptors or in the potentiating of IGF actions by facilitating the ligand–receptor interaction (Drop et al., 1992; Clemmons, 1993; Duan, 2002). mRNA for IGFBP-2, -3, -4 and -5 have been identified in leiomyoma and myometrium, and IGFBP-2, -3 and -4 have been detected in media of leiomyoma and myometrium explant cultures (Giudice et al., 1993; Vollenhoven et al., 1993, 1994; Van der Ven et al., 1996). The order of abundance of IGFBP mRNA expression in leiomyoma is IGFBP-4 >> IGFBP-3 >> IGFBP-5 > IGFBP-2, and IGFBP-1 and IGFBP-6 mRNA are undetectable in leiomyoma with no dependence on the in vivo estrogen status (Giudice et al., 1993). Although it has been suggested that IGFBP may modulate IGF actions in leiomyoma,
the precise roles of IGFBP in leiomyoma growth remain to be elucidated.

**Growth hormone**

Acromegaly increases the overall risk of neoplasms (De Menis et al., 1999). On the basis of high prevalence of leiomyomata in patients with acromegaly, leiomyomata is thought to be an additional feature of the organomegalic syndrome associated with acromegaly (Cohen et al., 1998). Growth hormone (GH) receptor mRNA has been detected in leiomyoma and the surrounding myometrium (Sharara and Nieman, 1995). This suggests that the uterus is a target tissue for GH action (Sharara and Nieman, 1995). In a guinea-pig model, GH increases the amount of ER in the uterus (Bezecny et al., 1992). Although it has been hypothesized that GH stimulates the production of hepatic IGF-I, and both GH and IGF-I act to promote the uterine growth (Hull and Harvey, 2001), the effect of GH on leiomyoma growth remains to be elucidated.

**Transforming growth factor-β**

Members of the transforming growth factor-β (TGFβ) family are pleiotropic cytokines with key roles in tissue morphogenesis and growth (Ingman and Robertson, 2002). TGFβ also acts to increase the expression of extracellular matrix (ECM) proteins such as fibronectin and collagen, and incorporate these proteins into the matrix (Ignotz and Massague, 1986; Ignotz et al., 1987). TGFβ1, -2 and -3 are abundant in mammalian reproductive tissues. Potential roles for TGFβ in reproductive tissues include gonad and secondary sex organ development, spermatogenesis and ovarian function, immunoregulation of pregnancy, embryo implantation, and placental development (Ingman and Robertson, 2002).

The expression of TGFβ1, TGFβ2 and TGFβ3 mRNA and TGFβ type I–III receptor mRNA has been reported in myometrium (Dou et al., 1996; Tang et al., 1997). However, the data on the expression of TGFβ in leiomyomata are conflicting, particularly in TGFβ1. A previous study showed that leiomyoma expressed higher levels of TGFβ1, TGFβ2 and TGFβ3 and TGFβ receptor type I and II mRNA than myometrium during the secretory phase of the cycle (Dou et al., 1996). Lee and Nowak (2001) reported that the levels of TGFβ1 mRNA were similar between leiomyoma and myometrium, whereas Arici and Sozen (2003) showed that TGFβ1 mRNA levels in myometrium were higher than in leiomyoma. As opposed to these results, Chegini et al. (1999) reported that leiomyoma had an increased expression of TGFβ1 mRNA compared with the adjacent myometrium.

With regard to TGFβ3, several authors agree that leiomyomata show the elevated levels of TGFβ3 mRNA compared with myometrium (Lee and Nowak, 2001; Arici and Sozen, 2003). Arici and Sozen (2000) have found that the highest level of TGFβ3 mRNA is observed in leiomyoma from the mid-secretory phase, suggesting the pivotal role of progesterone in the regulation of TGFβ3 expression. The effects of sex steroids on TGFβ expression have been documented. Diethylstilbestrol up-regulates mRNA of TGFβ1, -2 and -3 in the uterus of mouse (Takahashi et al., 1994). An injection of E2 enhances an induction of TGFβ2 mRNA in the uterus of adult ovariectomized mice, but an injection of progesterone has no effect on TGFβ2 mRNA levels, and co-injection of progesterone with E2 does not antagonize the E2-stimulated accumulation of TGFβ2 mRNA. Neither an injection of E2 nor progesterone exerts significant effects on TGFβ3 mRNA levels (Das et al., 1992). Moreover, the treatment with E2, medroxyprogesterone acetate, and E2 plus medroxyprogesterone acetate significantly increases TGFβ1 expression in leiomyoma cells. TGFβ1 increases the rate of [3H]thymidine incorporation into leiomyoma cells, and co-treatment of leiomyoma cells with E2 and TGFβ1 enhances the rate of [3H]thymidine incorporation (Chegini et al., 2002). Thus, TGFβ may be differently regulated by sex steroid hormones. TGFβ seems to modulate differently cell proliferation in leiomyoma and myometrium. TGFβ1 and TGFβ3 inhibit the DNA synthesis in myometrial cells, whereas leiomyoma cells do not show any growth inhibition in response to TGFβ1 and show an increase in the DNA synthesis when treated with TGFβ3 (Lee and Nowak, 2001). These findings suggest that alterations in the TGFβ system produce loss of sensitivity to the antiproliferative effects of TGFβ3, and increased expression of TGFβ3 may contribute to leiomyoma growth (Lee and Nowak, 2001).

Leiomyomas contain abundant quantities of ECM that consist primarily of collagen, proteoglycans and fibronectin. Leiomyomata have been reported to contain increased levels of mRNA for collagen type I and III (Stewart et al., 1994) and fibronectin (Arici and Sozen, 2000) relative to the adjacent myometrium. Treatment of cultured leiomyoma cells with TGFβ1 and TGFβ3 increases fibronectin mRNA levels (Arici and Sozen, 2000). Thus, TGFβ and their receptors are expressed at higher levels in leiomyoma than in myometrium, and the expression of TGFβ in leiomyoma may be under the control of sex steroids. TGFβ may play a role in the induction of cell proliferation and in the synthesis of ECM in leiomyoma cells.

**Platelet-derived growth factor**

Platelet-derived growth factor (PDGF) is a potent mitogen for smooth muscle cells and fibroblasts (Ross et al., 1986). PDGF constitutes a heterodimer of the A and B chain (PDGF-AB) and homodimers (PDGF-AA and PDGF-BB). PDGF exerts its biological effect by binding to specific cell surface receptor (PDGF-R).

Numerous studies have postulated a pivotal role for PDGF in leiomyoma growth. The immunoreactive PDGF β receptor has been detected in myometrial cells (Rossi et al., 1992) and leiomyoma cells (Palman et al., 1992). The co-expression of PDGF and PDGF-R is found in leiomyoma (Palman et al., 1992), suggesting an autocrine/paracrine role for PDGF in leiomyoma growth. Cultured leiomyoma cells have more PDGF-R sites than myometrial cells, but the receptor affinity is lower in leiomyoma than in myometrium (Fayed et al., 1989). The increase in immunoreactive PDGF expression and PDGF A-chain mRNA levels has been reported in gestational myometrium, indicating the association with uterine smooth muscle cell hypertrophy during pregnancy (Mendoza et al., 1990). However, two reports demonstrated no difference in the levels of PDGF A-chain mRNA (Mangrulkar et al., 1995) and PDGF B-chain mRNA (Bohm et al., 1990; Mangrulkar et al., 1995) between leiomyoma and myometrium. Nonetheless, PDGF has been reported to have the mitogenic activity in leiomyoma cells. PDGF stimulates the DNA synthesis (Fayed et al., 1989) and cell proliferation of leiomyoma cells (Arici and Sozen, 2003). Additionally, the interaction between estrogen and PDGF in leiomyoma has been demonstrated.
in vitro. PDGF is up-regulated by E₂, but down-regulated by anti-
estrogenic compound in cultured leiomyoma cells (Barbarisi et al., 2001). Barbarisi et al. (2001) have demonstrated that the addition
of antibodies directed against PDGF inhibits the mitogenic activity
present in conditioned media of cultured leiomyoma cells, and cell
treatment with the antiestrogenic compound correlates with the
disappearance of PDGF in the conditioned media. They suggest
the direct involvement of PDGF in leiomyoma proliferation. In
addition, GnRH agonist induces the decrease in immunoreactive
PDGF levels in leiomyoma, and decreased PDGF expression is
found to be significantly related to the shrinkage in uterine volume
(Di Lieto et al., 2002). These results lend supportive evidence for
a mitogenic action of PDGF on leiomyoma in vivo.

It has been reported that TGFβ1 stimulates the secretion
of PDGF at low concentrations, but down-regulates PDGF-R at high
concentrations in smooth muscle cells (Battegay et al., 1990). TGFβ1
also exerts a bimodal effect on the mitogenesis of
myometrial and leiomyoma cells. Arici and Sozen (2003) have
demonstrated that TGFβ1 induces a more prominent increase in
the DNA synthesis of leiomyoma cells compared with myometrial
cells at low concentrations and that the stimulatory effect
disappears at high concentrations. The authors speculate that
the growth stimulatory effect of TGFβ1 may be mediated through its
up-regulation of PDGF.

Angiogenic factors
It has been suggested that angiogenesis may play an important
role in the regulation of leiomyoma growth. Several angiogenic factors
have been studied in leiomyomas, including vascular endothelial
growth factor (VEGF), basic fibroblast growth factor (bFGF),
edothelin and adrenomedullin.

VEGF is a potent vascular endothelial cell mitogen (Ferrara et al., 1992) with five isoforms, termed VEGF-A, VEGF-B,
VEGF-C, VEGF-D and VEGF-E. The presence of VEGF mRNA
(Harrison-Woolrych et al., 1995) and VEGF-A protein (Gentry et al., 2001) has been detected in myometrium and leiomyoma.
Increased levels of VEGF mRNA are found in the secretory
myometrium compared with the proliferative myometrium, whereas VEGF mRNA levels in leiomyoma do not differ between
the proliferative and secretory phases of the menstrual cycle
(Harrison-Woolrych et al., 1995). VEGF mRNA levels in leiomyoma do not differ from those in myometrium (Harrison-
Woolrych et al., 1995). In addition, GnRH agonist does not affect
VEGF mRNA levels in leiomyoma (Harrison-Woolrych et al.,
1995). The authors suggest that VEGF expression in leiomyoma is
not regulated by ovarian steroids and that other angiogenic factors
may be more important than VEGF in angiogenesis associated
with leiomyoma growth.

Endothelin is a vasoconstrictor peptide with three distinct
peptide isoforms, termed endothelin-1, endothelin-2 and endothe-
lin-3. Endothelin can stimulate the DNA synthesis, cell division
and hypertrophy in monocytes and fibroblasts (Battistini et al.,
1993). mRNA encoding endothelin-1 and both the endothelin A
and endothelin B receptors have been detected in myometrium and
leiomyoma (Pekonen et al., 1994). Pekonen et al. (1994) have
demonstrated that endothelin A receptor mRNA is more abundant
in leiomyoma than in myometrium, whereas endothelin B receptor
mRNA is less abundant in leiomyoma. In contrast, other inves-
tigators indicate that only endothelin A receptors are present in
leiomyoma (Breuiller-Fouché et al., 1997; Honore et al., 2000).

Breuiller-Fouché et al. (1997) reported that this discrepancy might
be due to tumors at different stages of development being used. A
recent study has demonstrated that endothelin-1 cannot stimulate
the DNA synthesis on its own, but can potentiate the leiomyoma
cell growth effects of bFGF, EGF, IGF-I and IGF-II through a
protein kinase C-dependent pathway (Eude et al., 2001). This
suggests that endothelin-1 may act to promote leiomyoma growth
in an autocrine/paracrine fashion.

bFGF stimulates mitogenesis and differentiation of a variety of
cells, including fibroblasts and smooth muscle cells (Klagsbrun
and Dluz, 1993). The presence of bFGF, FGF receptor 1 and FGF
receptor 2 has been detected in myometrium and leiomyoma
(Mangrulkar et al., 1995; Anania et al., 1997; Wu et al., 2001).
One study demonstrated elevated levels of bFGF mRNA compared
with myometrium (Mangrulkar et al., 1995), but the other study
demonstrated the lack of differences in bFGF expression between
leiomyoma and myometrium (Wu et al., 2001). bFGF has been
shown to be mitogenic for both myometrial and leiomyoma cells,
but leiomyoma cells are found to be less responsive to the
mitogenic effects of bFGF (Rauk et al., 1995). These results
suggest that bFGF may not play a critical role in leiomyoma
growth.

An important role for adrenomedullin in leiomyoma angiogen-
esis has been implicated since this molecule is widely
expressed in leiomyoma and the expression of adrenomedullin correlates with vascular density and endothelial cell proliferation
in leiomyoma (Hague et al., 2000).

Sex steroid regulation of apoptosis-related factors

Bcl-2 protein

Apolipoprotein was first described as a morphological pattern of cell
death characterized by cell shrinkage, membrane blebbing and
chromatin condensation, culminating in cell fragmentation (Kerr et al., 1972). Despite the identification of genes necessary for
apoptotic cell death, the essential biochemical events in apoptotic
cell death remain largely unknown. Korsmeyer (1992) reported
that the bcl-2 proto-oncogene was unique among cellular genes in
its ability to block apoptotic cell death in multiple contexts. Over-
expression of bcl-2 in transgenic models leads to accumulation
of cells due to evasion of normal cell death mechanisms (McDonnell
et al., 1989). Accordingly, bcl-2 is thought to be a cell survival
gene.

Immunohistochemical analysis has revealed the presence of
Bcl-2 protein in leiomyoma and myometrium (Lu et al., 1993; Soini
and Paakko, 1996). Immunoreactive Bcl-2 protein is abundantly
detected in leiomyoma cells compared to that in
myometrial cells (Matsuo et al., 1997; Khurana et al., 1999; Wu
et al., 2002). Bcl-2 protein expression is shown to be much more
abundant in leiomyoma cells in the secretory phase of the
menstrual cycle than that in the proliferative phase (Matsuo et al.,
1997). By contrast, there is no apparent difference in the intensity
of immunostaining for Bcl-2 protein in myometrial cells between
the proliferative phase and the secretory phase of the menstrual
cycle (Matsuo et al., 1997).

Bcl-2 protein expression in leiomyoma cells is regulated by sex
steroid hormones. Cultured leiomyoma cells contain immuno-
reactive Bcl-2 protein with a molecular mass of ~26 kDa. Bcl-2 protein is abundantly present in leiomyoma tissue extracts, whereas Bcl-2 protein is undetectable in myometrial tissue extracts (Matsuo et al., 1997). The addition of progesterone remarkably increases the expression of 26 kDa Bcl-2 protein in cultured leiomyoma cells compared to that in control cultures, whereas the addition of E2 results in a somewhat lower expression of Bcl-2 protein in leiomyoma cells relative to that in untreated cultures (Figure 3) (Matsuo et al., 1997). In contrast, neither treatment with progesterone nor treatment with E2 affects the expression of Bcl-2 protein in myometrial cells (Matsuo et al., 1997). The molecular basis for progesterone action in the regulation of leiomyoma growth is not clear, but probably involves the progesterone-stimulated induction of Bcl-2 protein in leiomyoma cells. Since Bcl-2 protein has been shown to prolong cell survival by preventing apoptotic cell death, progesterone may act as a growth-promoting factor in regulating leiomyoma growth through up-regulating Bcl-2 protein.

Tumour necrosis factor-α

Tumour necrosis factor-α (TNFα) is one of the most versatile cytokines that are produced not only by activated macrophages but also by many types of cells in the female reproductive organs (Danforth and Sgagias, 1993; Terranova et al., 1995). The TNFα gene is located in the class 32 III region of the MHC, and codes for a 26 kDa membrane-bound form, which releases a 17.3 kDa soluble form upon cleavage from pro-TNFα (Beutler and Cerami, 1989; Camussi et al., 1991; Vassalli, 1992). Numerous studies have reported on the ability of TNFα to induce apoptosis of various cell types (Danforth and Sgagias, 1993; Pusztaí et al., 1993; Maruo, 1996; Maruo et al., 1999).

However, there is little information regarding the role for TNFα in leiomyoma growth and the effects of sex steroid hormones on TNFα expression in leiomyoma cells. Kurachi et al. (2001) was the first to demonstrate that immunoreactive TNFα expression is higher in leiomyoma cells than that in myometrial cells. Immunoreactive TNFα in leiomyoma cells is more abundant in the proliferative phase than in the secretory phase of the menstrual cycle, whereas there is no apparent difference in the immunointensity for TNFα in myometrial cells between the proliferative phase and the secretory phase of the menstrual cycle (Kurachi et al., 2001). Leiomyoma cells contain immunoreactive TNFα protein with a molecular mass of ~17.3 kDa. The addition of progesterone results in a remarkable decrease in the levels of 17.3 kDa immunoreactive TNFα in cultured leiomyoma cells compared with the control cultures (Figure 4) (Kurachi et al., 2001). The down-regulation of TNFα in cultured leiomyoma cells by progesterone is consistent with the immunohistochemical observation of lower expression of TNFα in leiomyomas in the secretory phase of the menstrual cycle. Treatment with E2 does not affect TNFα expression in cultured leiomyoma cells, whereas the concomitant treatment with E2 and progesterone slightly decreases the TNFα expression in leiomyoma cells relative to that in the treatment with E2 alone (Figure 4) (Kurachi et al., 2001). Thus, it seems likely that TNFα expression in leiomyoma cells may be regulated by progesterone.

The basic machinery for apoptosis constitutes a complex interaction between distinct pro-apoptotic and anti-apoptotic molecules. TNFα mediates apoptosis via the activation of caspases, whereas Bcl-2 protein inhibits TNFα-induced apoptosis in some cells (Haviv and Stein, 1999). Despite greater expression of immunoreactive TNFα protein in leiomyoma compared with myometrium (Kurachi et al., 2001), Wu et al. (2000) have reported no difference in apoptotic index between leiomyoma and...
myometrium or between the proliferative and secretory phases. By contrast, immunoreactive Bcl-2 protein is also abundantly expressed in leiomyoma cells compared to that in myometrial cells (Matsuo et al., 1997; Khurana et al., 1999; Wu et al., 2002b). Taking all these findings into account, it seems likely that Bcl-2 protein contributes to homeostatic control of leiomyoma growth by inhibiting TNFα-induced apoptosis in leiomyoma cells.

p53 protein

p53 gene is a tumour suppressor gene located on chromosome 17p. The wild-type p53 protein encoded by the p53 tumour suppressor gene is a nuclear phosphoprotein and is capable of suppressing the growth of a variety of cancer cells (Finlay et al., 1989; Chen et al., 1990; Malkin et al., 1990; Yin et al., 1992). It is widely recognized that p53 may be the most frequently mutated protein in human cancers, implying that an alteration of p53 is a fundamentally important step in genomic instability and susceptibility to neoplastic state transformation (Oren, 1992; Jung et al., 2001). p53 functions to regulate directly the expression of p21, Growth-arrest and DNA-damage inducible (GADD45), or Bax, which are involved in the control of cell cycle (Kastan et al., 1992) and programmed cell death (Oltvai et al., 1993; Miyashita et al., 1994; Miyashita and Reed, 1995). p53 binds to damaged DNA, repairs it, and induces cell cycle arrest at G1-phase or apoptosis (Lee and Berstein, 1993). The loss of p53 may contribute to tumour progression by arresting the cells in a relatively immature state (Oren, 1992). Therefore, p53 has been categorized as both a caretaker and gatekeeper tumour suppressor gene (Lane, 1992).

Although earlier studies detected little or no immunostaining for p53 in leiomyoma (Jeffers et al., 1995; Niemann et al., 1995; Wu et al., 2000), Gao et al. (2002) have demonstrated that the levels of p53 protein are higher in leiomyoma in the secretory phase than in the proliferative phase of the menstrual cycle by both immunohistochemistry and Western immunoblot analysis. However, there are no apparent differences in the p53 protein content between leiomyoma and the adjacent myometrium (Gao et al., 2002).

p53 has been shown to be regulated by sex steroid hormones. Treatment with GnRH agonist for 12–16 weeks up-regulates p53 protein content in leiomyoma compared with untreated leiomyoma (Gao et al., 2002). In addition, E2 treatment significantly decreases p53 protein content in cultured leiomyoma cells compared with untreated cultures, whereas either progesterone treatment or combined treatment with E2 and progesterone does not affect p53 protein content (Figure 5) (Gao et al., 2002).

Since p53 is a tumour suppressor gene that directly regulates the growth of tumours by inhibiting tumour cell growth or promoting cell death (Kastan et al., 1992; Oltvai et al., 1993; Miyashita et al., 1994, 1995), E2 may stimulate leiomyoma growth in part by decreasing the p53 protein levels and suppressing normal p53 functions. Conversely, treatment with progesterone alone or E2 plus progesterone does not affect p53 protein content in cultured leiomyoma cells. These findings suggest that progesterone has no effect on p53 protein content or that progesterone blocks or inverses the effect of E2 on p53 protein expression in leiomyoma cells.

It is noteworthy that p53 protein may be highly unstable in the majority of human cancers (Oren, 1992; Jung et al., 2001), the alterations in p53 protein content may be determined not only by p53 protein expression but also its degradation in leiomyoma cells.

p53 degradation is commonly controlled by the ubiquitin-proteasome pathway (UPP), which is the major system for the rapid degradation of some regulatory proteins in the eukaryotic cells (Ciechanover, 1994). Although there is no apparent evidence suggesting that the UPP-dependent degradation is involved in the alterations in p53 protein content in leiomyoma cells, many studies have demonstrated that p53 protein is degraded and controlled through the UPP in other cell types (Ciechanover et al., 1991; Crook et al., 1996). If p53 is normal, it guards the genome against somatic mutations that may initiate cancer (Finlay et al., 1989; Chen et al., 1990; Malkin et al., 1990; Yin et al., 1992). If p53 is inactivated, however, it is unable to prevent increased genetic instability and lacks anti-oncogenic function (Nigro et al., 1989; Vogelstein and Kinzler, 1992).

GnRH agonist therapy

GnRH agonist suppresses pituitary gonadotrophin biosynthesis by decreasing the number and sensitivity of GnRH receptors, which leads to the reduction in plasma sex steroid hormone levels. GnRH agonist therapy is an effective treatment for leiomyomas, but the molecular events necessary for GnRH agonist-induced reduction in the size of leiomyomas are not well understood. GnRH agonist has been shown to inhibit the expression of several growth factors involved in the regulation of leiomyoma growth, including the

Figure 5. Effects of sex steroids on p53 protein content in cultured leiomyoma cells, as assessed by Western immunoblot analysis. (A) The p53 protein content was decreased in leiomyoma cells treated with 17β-estradiol (E2) (10 ng/ml) compared with that in untreated cells in control cultures, whereas treatment with either progesterone P4 (100 ng/ml) or E2 (10 ng/ml) plus progesterone (100 ng/ml) showed no apparent effect on p53 protein content in cultured leiomyoma cells. (B) β-actin as a loading control. (C) Relative content assessed by densitometric analysis refers to the ratio of p53 content observed relative to that in control cultures. *P < 0.05 versus p53 content in leiomyoma cells in untreated control cultures. Adapted from Gao et al. (2002). Reproduced by permission of The Endocrine Society. © Copyright 1997, 1998, 2001 and 2002. The Endocrine Society.
decrease in the binding of EGF to leiomyoma (Lumsden et al., 1988) and immunostaining for EGF-R in leiomyoma (Leone et al., 1991), the decrease in the secretion of IGF-I and IGF-II by explant cultures of leiomyoma (Rein et al., 1990) and IGF-I mRNA levels in leiomyoma (Wu et al., 2002a), and the decrease in the levels of TGFβ and its receptors (Dou et al., 1996; Di Lieto et al., 2003). However, conflicting results exist as to whether GnRH agonist induces apoptosis of leiomyoma cells (Higashijima et al., 1996; Burroughs et al., 1997; Mizutani et al., 1998; Huang et al., 2002). Some investigators have reported that apoptosis is increased in leiomyoma cells obtained from patients treated with GnRH agonist compared to those from non-treated patients (Higashijima et al., 1996; Mizutani et al., 1998), whereas Huang et al. (2002) failed to detect the increase in apoptosis. Mizutani et al. (1998) have reported a transient increase in apoptosis of leiomyoma cells at the 4th week of GnRH agonist therapy and low levels of apoptosis at all times other than that week. In addition, Higashijima et al. (1996) have observed increased apoptosis of leiomyomas in patients treated with GnRH agonist compared to the non-treated group, suggesting that the induction of apoptosis may be a mechanism of the effect of GnRH agonist in leiomyomas. Actually, Wang et al. (2002) have reported that GnRH agonist directly inhibits the growth of cultured leiomyoma cell proliferation and induces apoptosis in association with the increase in Fas expression and the induction of Fas ligand. Fas and Fas ligand are typical members of the TNF receptor and TNF ligand family. Since the engagement of Fas by Fas ligand triggers a cascade of subcellular events that result in apoptosis, Fas-Fas ligand system may participate in GnRH agonist-induced apoptosis of cultured leiomyoma cells. On the other hand, p53 activation transiently increases surface Fas expression by transport of Fas from the Golgi complex, and then induces apoptosis in human vascular smooth muscle cells (Bennett et al., 1998). p53 protein content was shown to be significantly increased in leiomyoma during GnRH agonist therapy for 16 weeks compared with that in untreated leiomyoma (Gao et al., 2002). Therefore, it is likely that GnRH agonist increases the expression of Fas on the membrane surface mediated through activation of p53 protein, and then plays a role in induction of apoptosis by Fas/Fas ligand system. Consequently, the increase in p53 protein content, Fas and Fas ligand during GnRH agonist therapy may explain one of the molecular bases for GnRH agonist-induced apoptosis in cultured leiomyoma cells. In contrast, in cell lines derived from the Eker rat model of uterine leiomyoma, estrogen-depleted medium and anti-estrogen tamoxifen did not induce apoptosis despite a significant reduction in cell numbers and the arrest of cell proliferation (Burroughs et al., 1997). Huang et al. (2002) have indicated that GnRH agonist therapy down-regulates the expression of Fas ligand and caspase 3 with no concomitant increase in apoptosis of leiomyomas. These discrepancies may be attributable to a possible direct effect of GnRH agonist on leiomyoma cells and the differences between in vitro and in vivo experiments.

Recent studies have implicated an autocrine/paracrine role for GnRH in leiomyoma cells. Specific binding sites for GnRH are present in leiomyoma (Wiznitzer et al., 1988), and GnRH and GnRH receptor mRNA are expressed in myometrial and leiomyoma cells (Chegini et al., 1996; Wang et al., 2002). These results support the concept that GnRH agonist may directly exert its effect on leiomyoma cells. In fact, the inhibitory effects of GnRH agonist treatment on the growth of cultured leiomyoma cells are dose-dependent (Wang et al., 2002). Two hypotheses can be proposed to explain the direct effect of GnRH agonist on leiomyoma growth. First, the prolonged exposure to GnRH agonist may not result in the internalization of GnRH receptor or the desensitization to GnRH agonist stimulation in leiomyoma cells. Second, GnRH agonist may up-regulate the expression of GnRH receptor in leiomyoma cells in a dose-dependent manner.

Conclusions

This review has focused on the effects of sex steroid hormones on the expression of several local growth factors and apoptosis-related factors in leiomyoma cells. A great deal of evidence supports the concept that ovarian sex steroid hormones, growth factors and apoptosis-related factors may play critical roles in the regulation of leiomyoma growth. In addition, growth factors have been shown to be involved in the promotion of leiomyoma cells under the control of sex steroids. However, the precise molecular mechanisms by which sex steroids regulate and cooperate with growth factors in leiomyoma cells remain to be elucidated. On the basis of the data obtained, a possible cross-talk between sex steroid hormones, growth factors and apoptosis-related factors in the regulation of uterine leiomyoma cell growth is summarized as shown in Figure 6. E2 may contribute to the promotion of leiomyoma cell growth through up-regulating EGF-R, TGFβ1, TGFβ3 and PDGF, and to the survival of leiomyoma cells through up-regulating wild type p53. On the other hand, progesterone may contribute to the promotion of leiomyoma cell growth through up-regulating EGF, TGFβ1 and TGFβ3, and the survival through up-regulating Bcl-2 expression and down-regulating TNFα expression in leiomyoma cells. The widely accepted view for the net effects of progesterone on leiomyoma growth is in favour of the mitogenic activity for leiomyoma cells. However, progesterone may also exert an inhibitory effect on leiomyoma growth and survival through down-regulating IGF-I expression. This suggests that progesterone may have dual actions on leiomyoma growth: one is the action to stimulate and the other is the action to inhibit leiomyoma growth.

Some clinical evidence supports the concept that progesterone may have dual actions on leiomyoma growth. Since the circulating...
concentrations of E2 and progesterone are considered to be the major determinants responsible for leiomyoma growth, most clinicians believed until recently that uterine leiomyomas increase in size during pregnancy in response to the increased circulating concentrations of E2 and progesterone. However, leiomyomas do not necessarily increase in size during pregnancy despite the increased circulating concentrations of E2 and progesterone. Lev-Toaff et al. (1987) reported that leiomyomas only occasionally increased in size during the first trimester, and that very few continued to grow during the course of pregnancy. Phelan (1995) also described that most leiomyomas identified early in pregnancy remained the same size or even shrank over the course of pregnancy. On the other hand, the use of levonorgestrel-releasing intrauterine system (LNG-IUS) is effective for a long-term management of menorrhagic women with uterine leiomyomas because of a striking reduction in menstrual bleeding volume (Maruo et al., 2001a). Although the LNG-IUS insertion uniformly causes the atrophic changes of the endometrium associated with decreased proliferation and increased apoptosis (Maruo et al., 2001b), the effect of LNG-IUS on the size of leiomyomas is remarkably varied: the size of uterine leiomyomas during the use of LNG-IUS is noted to increase, remain the same or decrease in each one-third of the cases examined (Maruo et al., 2001b). The individual variations of the size of leiomyomas during the use of LNG-IUS may be dependent on the local autocrine/paracrine growth factor conditions around each leiomyoma. These clinical findings may be explained at least in part by the dual actions of progesterone on leiomyoma growth.

It seems likely that the interaction between Bcl-2 protein and TNFα in leiomyoma may result in the predominance of anti-apoptotic pathway due to the inhibitory effect of Bcl-2 protein on TNFα-induced apoptosis. Moreover, recent studies have emphasized the importance of the cross-talk between ER and PR signalling pathways in leiomyoma growth because the interrelationship between both the receptors can modulate biological responses of sex steroid hormones.

Intracellular signalling pathways of growth factors have recently been extensively examined. Interestingly, Xu et al. (2003) have demonstrated that myometrial cells and leiomyoma cells express Smads, TGFβ receptor intracellular signalling molecules, which are differently expressed, induced, activated by TGFβ and are altered as a result of GnRH agonist treatment. However, the interaction between sex steroids and intracellular signalling pathways of growth factors in leiomyoma remains to be investigated. Further studies are warranted to define the molecular bases of the cross-talk between sex steroids and their receptors and growth factors in regulating uterine leiomyoma growth.

Acknowledgements

The authors wish to thank the postgraduate colleagues and clinical fellows who performed the research in the laboratory of the Department of Obstetrics and Gynecology of Kobe University Graduate School of Medicine. Special thanks go to Dr T.Samoto, Y.Shimomura, O.Kurachi, Z.Gao, Y.Wang, T.Yamada and S.Nakago. The research was supported in part by Grants-in-aid for Scientific Research no. 12877263 from the Japanese Ministry of Education, Science and Culture and by Ogaya-Donation Foundation of the Japan Association of Maternal Welfare.

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