Expression of Proliferative and Preapoptotic Molecules in Human Myometrium and Leiomyoma Throughout the Menstrual Cycle

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*The pathogenesis of leiomyoma may be related to an imbalance in the interaction of sex steroids with paracrine growth factors that may control the modulation of mitogenesis and local immunity. The authors investigate the temporal and spatial expression of proliferative and preapoptotic molecules that may participate in the modulation of myometrial function and leiomyoma pathogenesis. Immunohistochemistry and Western blot analysis are used to investigate Fas ligand (FasL), phosphatase and tensin homolog deletion on chromosome 10 (PTEN), and proliferating cell nuclear antigen (PCNA) expression in myometrium and leiomyoma. Western blot results show that in the secretory phase, FasL expression is 1.8-fold and 2.3-fold higher compared with the proliferative phase in the myometrium and leiomyoma, respectively (*P *= .022 and .047, respectively). A paired comparison between myometrium and leiomyoma reveals higher FasL expression in the leiomyoma (*P *= .003). On the contrary, when compared with the secretory phase, PCNA expression during the proliferative phase is 4.6-fold and 3.7-fold higher in the myometrium and leiomyoma, respectively (*P *= .041 and .034, respectively). A paired comparison between myometrium and leiomyoma reveals higher PCNA expression in the leiomyoma. Furthermore, lower PTEN expression is detected in the leiomyoma compared with the myometrium (*P *< .032). Immunohistochemistry results reveal that FasL, PTEN, and PCNA are expressed in the myometrium and leiomyoma, consistent with the results from the Western blot analysis. The results suggest that FasL, PTEN, and PCNA may be involved in the pathophysiology of leiomyoma. A higher FasL level in the leiomyoma is likely to correspond to suppression of local immunity by inducing apoptosis of immune cells, while a higher level of PCNA and a lower level of PTEN may be related to increased mitogenesis and decreased apoptosis in leiomyoma.*

Key words: Fas ligand, PTEN, PCNA, leiomyoma, myometrium.

The myometrium consists of 3 poorly distinguishable layers of longitudinally, obliquely, and circularly arranged smooth muscle fibers that undergo hypertrophy and hyperplasia during pregnancy. These physiologic aspects of myometrium emerge in response to hormonal changes and paracrine interactions.[1]

Leiomyoma, the benign neoplasm of myometrium, is the most common pelvic tumor and is diagnosed clinically in at least 20% of women of reproductive age.[2,3] In 20% to 50% of cases, leiomyoma causes clinically relevant symptoms requiring either a surgical or medical treatment.[4] Transition to the postmenopausal period and the use of gonadotropin-releasing hormone (GnRH) agonist treatment cause significant reduction in the leiomyoma size, suggesting that the size and tumor growth rate are associated with the presence of sex steroid hormones.[5] Recent findings on the autocrine and paracrine interactions of the growth factors in leiomyoma suggest that molecular cross-talk between these factors and sex steroids may be involved in the development and/or progression

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of leiomyoma.[6] Leiomyoma also contains abundant extracellular matrix deposits besides the proliferated smooth muscle cells.[7,8]

Apoptosis, programmed cell death, is an essential regulator of cell turnover in tissues during prenatal and postnatal life. Fas ligand (FasL) is a 37-kDa protein that belongs to the tumor necrosis factor (TNF) superfamily. As a mediator of apoptosis in differentiated cells and embryonic development, it interacts with its receptor Fas and induces apoptosis in Fas-bearing cells in an autocrine or paracrine manner.[9] An increased level of FasL causes apoptosis in immune cells and may regulate local immune response in this part of the myometrium.

As a downregulatory protein of apoptotic signaling, the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) gene encodes a 403-amino-acid tyrosine phosphatase that inhibits the phosphatidylinositol 3-kinase/Akt (PIK3-Akt) signaling pathway. Akt signaling is a critical determinant for controlling cell growth, proliferation, and survival.[10] PTEN is frequently inactivated in many common malignancies, including glioblastoma and endometrial and prostate cancers. It downregulates cell growth and proliferation. There is a strong correlation between its phosphatase and tumor suppressor activity that has been demonstrated previously in many advanced cancers in vitro.[11,12] Therefore, PTEN plays important roles in cell growth and survival. PTEN may be downregulated in leiomyoma, which in turn causes activation of PIK3-Akt signaling and increases the cell survival molecules and genes in leiomyoma cells.

Proliferating cell nuclear antigen (PCNA) is a 36-kD acidic nuclear protein, found only in the nucleus of dividing (proliferating) cells. It is involved in many aspects of DNA replication. In particular, the amount of PCNA increases during the late G1 phase, reaching a nadir in the S phase, and then declining during the G2 phase.[13] Recent studies have shown its striking ability to interact with multiple partners, which are involved in several metabolic pathways, including Okazaki fragment processing, DNA repair, translation, DNA synthesis, DNA methylation, chromatin remodeling, and cell cycle regulation.[4,14]

Many uterine-related diseases are closely related to changes in cell proliferation and apoptosis. A recent study has suggested that oncoprotein p53, MDM2, and p21Waf1 may play a role in the regulation of endometrioma cell growth but not in adenomyosis.[15] Goumenou et al[16] have shown that endometriotic glandular and stromal cells express different levels of proapoptotic molecules such as Bcl-2 and Bax, correlating with the apoptosis rates observed in these cells.

The pathogenesis of leiomyoma may be related to an imbalance in the interaction of sex steroids with paracrine growth factors and cytokines that may control the modulation of mitogenesis, apoptosis, and local immunity. Our hypothesis is that differences in the temporal and spatial expression of proliferative and preapoptotic molecules may participate to the pathogenesis of leiomyoma. To test our hypothesis, we assess the expression of 2 major regulatory proteins of apoptosis (FasL and PTEN) and 1 endogenous marker of proliferation (PCNA) in leiomyoma and its paired homologous myometrium from premenopausal women throughout the menstrual cycle using immunohistochemistry and Western blot analysis.

METHODS

Tissue Collection

Uterine leiomyoma tissues and the adjacent myometrium were obtained from premenopausal women with regular menstrual cycles who underwent hysterectomy for uterine leiomyoma at Yale New Haven Hospital. The study protocol for the collection of surgical specimens was approved by the Human Investigation Committee of Yale University. Informed consent was obtained from each patient before surgery for the use of uterine tissues. Patients had received no hormonal therapy for at least 3 months before the surgery. The age of patients ranged from 29 to 54 years, with a mean age of 44.1 years. Endometrial tissues were also obtained from the extirpated uterus, and the day of the menstrual cycle was determined by histological examination of the endometrium according to the method of Noyes et al.[17] Tissues ($N = 40$) were divided into 2 groups: Group 1 consists of myometrium of women with leiomyoma ($n = 20$), and group 2 consists of paired leiomyoma tissues of group 1 ($n = 20$). The peripheral parts of leiomyoma tissues (the outer 5 mm under the tumor capsule) and adjacent myometrium were dissected from endometrial cell layers. Then, leiomyoma and myometrium were processed separately. A piece of each tissue was embedded in cryomatrix for frozen sectioning, and the remaining part was used for protein extraction using radioimmune precipitation (RIPA) buffer, as described previously.[18]

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Western Blot Analysis

Total protein from myometrial and leiomyoma tissues was extracted with RIPA protein extraction buffer (50 mmol/L Tris-HCl [pH 7.4], 150 mmol/L NaCl, 1% Triton X-100, 0.5% sodium deoxycholic acid, 0.1% SDS, and 1% protease inhibitor cocktail). The protein concentration was determined by a detergent-compatible protein assay (Bio-Rad, Hercules, CA). Samples (20 μg) were loaded on 7.5% Tris-HCl Ready Gels (Bio-Rad), electrophoretically separated and electroblotted onto nitrocellulose membrane (Bio-Rad). The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBS-T) for 1 hour to reduce nonspecific binding. Subsequently, the membrane was incubated for 1 hour with monoclonal mouse antihuman FasL (1:1500; Transduction Laboratories, New Castle, UK), monoclonal mouse antihuman PTEN (1:1000; Cell Signaling, Beverly, MA), and monoclonal mouse anti-PCNA (1:2000; Sigma-Aldrich, St Louis, MO) antibodies. After several washings with TBS-T, membranes were further incubated with peroxidase-labeled antimouse IgG for 1 hour. Membranes were then washed 6 times with TBS-T for 1 hour, and the reaction was visualized by light emission on film (Amersham Biosciences, Buckinghamshire, UK) using enhanced chemiluminescence peroxidase substrate (Amersham Biosciences). After stripping the membrane with Western blot stripping buffer (Pierce, Rockford, IL), the same steps were performed for mouse antihuman glyceraldehyde phosphate dehydrogenase (GAPDH) monoclonal antibody (Santa Cruz Biotechnology, CA) to confirm equal loading of proteins in each lane. Autoradiographic bands for FasL, PTEN, PCNA, GADPH, or β-actin were quantified by a digital imaging and analysis system (AlphaEase; Aplha Innotech Corp, San Leandro, CA) and a laser densitometer (Molecular Dynamics, Inc, Sunnyvale, CA).[18] Briefly, all bands were scanned at the same magnification and resolution. Thereafter, an equal reading quadrangle (0.2 cm width × 0.8 cm length) was applied to read each band's intensity, and results were recorded as arbitrary densitometric units. FasL, PTEN, and PCNA expression was then normalized by dividing the arbitrary densitometry units for FasL, PTEN, and PCNA by those for GAPDH or β-actin for each band.

Immunohistochemistry

Frozen sections from snap-frozen samples were obtained at 7-μm thickness and fixed in cold methanol (–20°C for 10 minutes) and dried for 20 minutes at room temperature. After several washings in distilled water and then 3 washings in TBS (pH 7.4) for 10 minutes, endogenous peroxidase activity was quenched with 3% H_2O_2 (0.6 mL H_2O_2 and 5.4 mL methanol) for 10 minutes and rinsed in TBS-T. Sections were then incubated with monoclonal mouse antihuman FasL (1:200; Transduction Laboratories) monoclonal mouse antihuman PTEN (1:100; Cell Signaling), and monoclonal mouse anti-PCNA (1:100; Sigma-Aldrich) antibodies for 30 minutes at room temperature in a humidified chamber. For negative controls, primary antibodies were replaced by their nonimmune isotypes. The sections were washed 3 times for 5 minutes with TBS, and biotinylated antimouse antibody (1:400; Vector Labs, Burlingame, CA) was then added at a 1:400 dilution for 30 minutes at room temperature. After washing 3 times with TBS, sections were incubated with the avidin-peroxidase kit (Vector Labs). Diaminobenzidine (3, 3-diaminobenzidine tetrahydrochloride dihydrate; Vector Labs) was used as the chromogen and mounted with Permount mounting medium (Fisher Chemicals, Springfield, NJ) on glass slides. The immunoreactivity intensity for FasL, PCNA, and PTEN was semiquantitatively evaluated using the following intensity categories: 0, no staining; 1+, weak but detectable staining; 2+, moderate or distinct staining; and 3+, intense staining.

Statistical Analyses

Statistical analyses were performed using ANOVA and Student *t* test or paired *t* test, where appropriate. Statistical significance was set at $P < .05$. If the data from Western blot results were not normally distributed (as determined by the Kolmogorov-Smirnov test), pairwise multiple comparisons were analyzed with nonparametric ANOVA on ranks (Kruskal-Wallis test) followed by the post hoc Student-Newman-Keuls test. Statistical calculations were performed using SigmaStat for Windows, version 3.0 (Jandel Scientific Corp, San Rafael, CA).

RESULTS

Increased Level of FasL Expression in Leiomyoma During the Secretory Phase

To compare the level of FasL expression, we used Western blot analysis using myometrium and leiomyoma protein extracts. We observed cycle-dependent changes for the expression of FasL. Myometrial samples from the secretory

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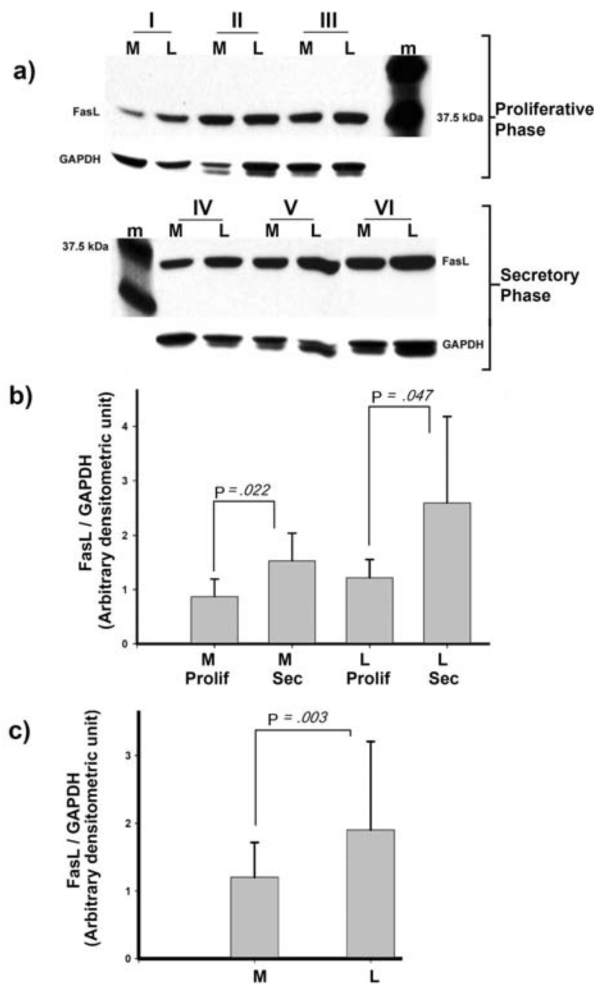

Figure 1. Analysis of Fas ligand (FasL) expression in myometrial and leiomyoma protein extracts throughout the menstrual cycle using Western blot. Representative immunoblot bands from proliferative and secretory tissues are seen (I-IV: the number of paired myometrium and leiomyoma for each patient) (a). Results were semiquantified and presented according to menstrual cycle phases (proliferative myometrium/ leiomyoma, n = 10; secretory myometrium/leiomyoma, n = 10) (b) and in a cycle-independent manner (myometrium/leiomyoma, n = 20) (c). FasL levels were normalized to glyceraldehyde phosphate dehydrogenase (GAPDH) protein levels. Prolif, proliferative phase; Sec, secretory phase; M, myometrium; L, leiomyoma; m, molecular weight marker. Bars represent the mean ± SEM. *$P < .05$.

phase expressed higher FasL than that in samples from the proliferative phase (Figure 1a, b; $P = .022$). Leiomyoma samples from the secretory phase revealed a higher FasL expression as well (Figure 1a, b; $P = .047$). Semiquantitative analysis of Western blot results revealed that during the secretory phase, FasL expression was 1.8-fold and 2.3-fold higher compared with the proliferative phase in the myometrium and leiomyoma, respectively (Figure 1b). Moreover, comparison of myometrium tissues with their paired leiomyoma showed a 1.8-fold higher FasL expression in the leiomyoma in the secretory phase (Figure 1b; $P = .003$).

PCNA Expression in Human Myometrium and Leiomyoma Throughout the Menstrual Cycle

To compare the proliferative activity, we evaluated the expression of PCNA using Western blot analysis in myometrial and leiomyoma protein extracts. The analysis of Western blot results showed that PCNA expression has cycle-dependent changes in myometrium and leiomyoma (Figure 2a, b). Myometrial samples from the secretory phase revealed lower PCNA expression than that in myometrium samples from the proliferative phase (Figure 2a, b; $P = .041$). Similarly, PCNA expression was higher in the proliferative phase leiomyoma tissues compared with the secretory phase leiomyoma tissues (Figure 2a, b; $P = .034$). PCNA expression during the proliferative phase was 4.6-fold and 3.7-fold higher in the myometrium and leiomyoma, respectively, compared with secretory phase tissues (Figure 2b). Moreover, paired comparison of myometrial tissues with their autologous leiomyomas showed a 2.1-fold higher PCNA expression in the leiomyoma (Figure 2c; P = .007).

Regulation of PTEN Expression in Human Myometrium and Leiomyoma Throughout the Menstrual Cycle

To reveal whether PTEN is involved in the pathogenesis of leiomyoma, PTEN levels were compared semiquantitatively between leiomyoma tissues and their autologous myometrial tissues using Western blot analysis. The analysis of Western blot results showed that PTEN expression did not change significantly in the myometrium and leiomyoma throughout the cycle (Figure 3a, b). On the other hand, the paired analysis revealed that the PTEN levels were significantly lower in leiomyoma when compared with those in the myometrium (Figure 3c; $P = .032$). Moreover, PTEN expression was significantly decreased in proliferative phase leiomyomata samples but not in secretory phase leiomyomata samples compared with their autologous myometrium (Figure 3b; $P = .029$).

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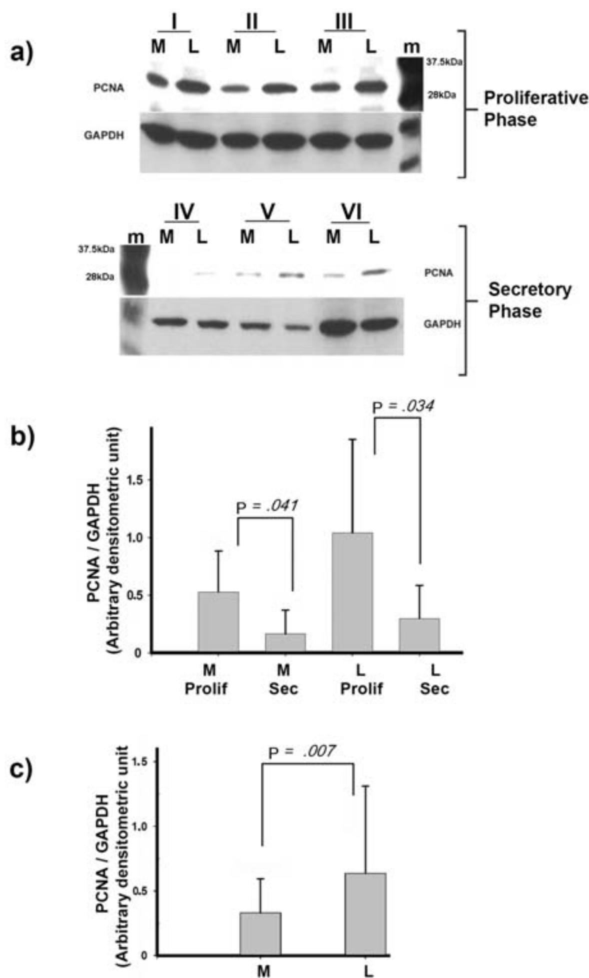

Figure 2. Analysis of proliferating cell nuclear antigen (PCNA) expression in myometrial and leiomyoma protein extracts throughout the menstrual cycle using Western blot. Representative immunoblot bands from proliferative and secretory tissues are seen (I-IV: the number of paired myometrium and leiomyoma for each patient) (a). Results were semiquantified and presented according to menstrual cycle phases (proliferative myometrium/leiomyoma, n = 10; secretory myometrium/leiomyoma, n = 10) (b) and in a cycle-independent manner (n = 20) (c). PCNA levels were normalized to glyceraldehyde phosphate dehydrogenase (GAPDH) protein levels. Prolif, proliferative phase; Sec, secretory phase; M, myometrium; L, leiomyoma; m, molecular weight marker. Bars represent the mean ± SEM. *$P < .05$.

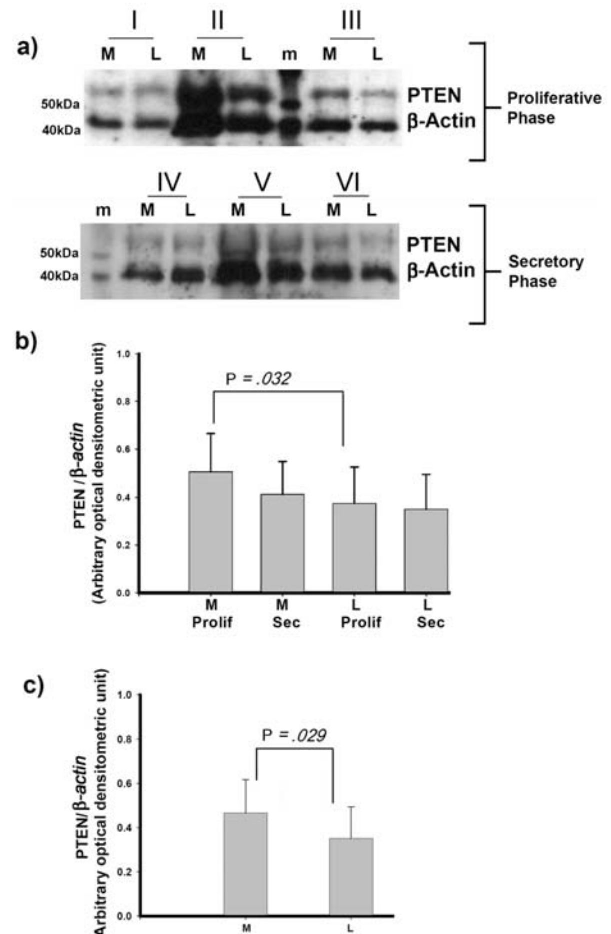

Figure 3. Western blot analysis of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) expression in myometrium and leiomyoma. Representative immunoblot is presented (I-IV: the number of paired myometrium and leiomyoma for each patient) (a). Results were semiquantified and presented according to menstrual cycle phases (proliferative myometrium/leiomyoma, n = 10; secretory myometrium/leiomyoma, n = 10) (b) and in a cycle-independent manner (n = 20) (c). PTEN levels were normalized to β-actin protein level. Prolif, proliferative phase; Sec, secretory phase; M, myometrium; L, leiomyoma; m, molecular weight marker. Bars represent the mean ± SEM. *$P < .05$.

Cellular Expression of FasL, PTEN, and PCNA in Human Myometrium and Leiomyoma

Immunohistochemistry results revealed that FasL, PTEN, and PCNA are expressed in smooth muscle, vascular, and intermediate cells of the myometrium (Figure 4a-c). Leiomyoma samples revealed a higher immunoreactivity for FasL and lower immunoreactivity for PTEN compared with those of paired myometrium samples (Figure 4a, b, d, e). The number of PCNA-immunopositive smooth muscle cells was higher in the leiomyoma than in the paired myometrium (Figure 4c, f).

DISCUSSION

Leiomyoma is a steroid-dependent benign neoplasm of myometrium. The pathogenesis of leiomyoma most likely

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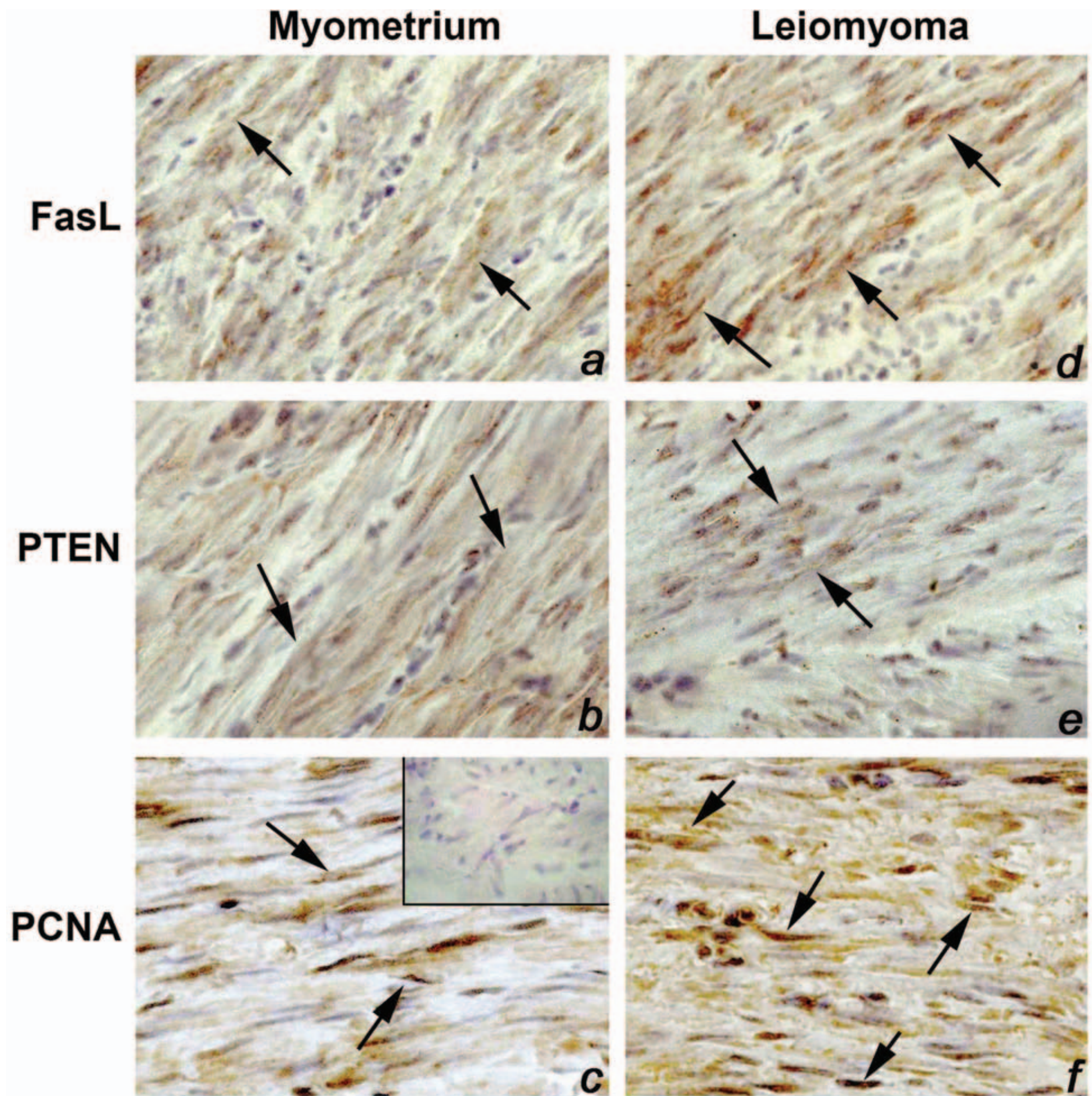

Figure 4. Representative micrographs of Fas ligand (FasL), phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and proliferating cell nuclear antigen (PCNA) immunostaining in myometrium and leiomyoma. Immunopositive myometrial (n = 6) and leiomyoma (n = 6) smooth muscle cells are indicated with arrows for each protein. While FasL (a, d) and PTEN staining (b, e) was in the cytosol, PCNA was mostly nuclear (c, f). Higher FasL and PCNA immunoreactivity is seen in leiomyoma (d, f) compared with their paired myometrium (a, c), while a decreased level of PTEN expression in leiomyoma (e) is seen compared with the paired myometrium (b). A negative control staining is presented in the inset (c).

involves genetic alternations in normal myometrial cells and complex interactions of sex steroids with cytokines and growth factors, resulting in modulation of the local immune response.[19] We found that myometrial and leiomyoma cells show cycle-dependent changes of the expression of major apoptosis regulatory proteins (FasL and PTEN) and of an endogenous marker of cell proliferation protein (PCNA), suggesting direct or indirect sex steroid–dependent regulation of these proteins.

The growth of leiomyomas arising from uterine smooth muscle cells is modulated by circulating steroid hormones and is associated with adequate levels of estrogen and

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progesterone. The inhibition of ovarian hormone production by endocrine therapy often results in the regression of these tumors, but the role of apoptosis in this process has not been elucidated.[20] Because of the expansive nature of some uterine leiomyomas in the absence of high mitotic activity, we were interested in the role of altered programmed cell death (apoptosis) versus cell proliferation as a mechanism of uterine leiomyoma growth. There are at least 2 pathways that activate apoptosis. The first mechanism involves the activation of a group of TNF receptors, such as Fas (ligand-receptor pathway). The second (exogenous stimulus pathway) is a parallel mitochondria-dependent route activated by physiological stimuli (lack of growth factors, changes in hormonal environment) and/or environmental stimuli (exposure to cytotoxic compounds).[21]

Interaction of FasL with its receptor, Fas, induces apoptosis through autocrine and paracrine signaling. Immunohistochemical study revealed that in the uterine smooth muscle cells, Fas and FasL predominantly exist in the cytoplasm instead of on the cell membrane, as seen in activated lymphocytes or epithelial cells.[22] These findings suggest that the coexpression of Fas and FasL in uterine smooth muscle cells is unlikely to make them immune privileged or to regulate cell turnover or growth because of their cytosolic localizations.[22] The finding that GnRH agonists directly inhibit the growth of cultured leiomyoma cell proliferation and induce apoptosis in association with an increase in the Fas expression and the induction of FasL strongly supports the idea that Fas-FasL interaction may be functional in these cells.[23,24] Our results reveal an increased expression of FasL in the secretory phase compared with that in the proliferative phase in both myometrial and leiomyoma tissues. Compared with myometrium, leiomyoma expressed significantly higher FasL protein in only the secretory phase samples, and there was no significant difference between the myometrial and leiomyoma samples in the proliferative phase. Previously, Selam et al[25] showed that FasL expression increased gradually through the mid and late proliferative phases in both endometrial stromal and glandular cells. Strong FasL expression was observed throughout the late-proliferative and secretory phases, and estradiol and progesterone stimulated FasL mRNA and protein expression in these cells.[25] Similar to this observation, we suggest that FasL may induce apoptosis in activated immune cells, such as macrophages and natural killer cells infiltrating the leiomyoma tissues. This in turn could suppress the immunologic response developed against leiomyoma cells.

Bourlev et al[26] suggested that the growth of leiomyoma mainly occurs in the peripheral parts of the tumor during the secretory phase of the menstrual cycle, as the mitotic index was significantly higher in the peripheral part than in the central part of the leiomyomas in the secretory phase. They also showed that the apoptotic index was significantly higher in the peripheral part compared with the central part during the proliferative phase. With these results, they suggested that leiomyoma growth mainly occurs during the secretory phase. Moreover, they observed no significant difference for either the proliferation index or apoptotic index when the proliferative and secretory cycle phases were compared. Kawaguchi et al[27] suggested that the mitotic activity of leiomyomas rises at the beginning of the luteal phase and remains high until menses, which points to the pivotal role of progesterone in the cellular proliferation of these tumors. Previous studies[28,29] have investigated the same proliferative activity through the expression of PCNA. These studies demonstrated that leiomyomas proliferated most actively during the luteal phase of the cycle. But Zaslawski et al[30] suggested that the proliferative activity of the leiomyoma should be estimated using mitotic markers.[30] They suggested that the expressions of PCNA and Ki-67 antigens were not dependent on the phase of the menstrual cycle and that natural variations in the serum levels of sex steroids did not significantly affect the synthesis of these proteins or their mitotic activity in the target cells.[30] Our finding suggests that higher mitotic indices in leiomyoma samples may be due to the increase of PCNA expression in these samples compared with myometrial samples. However, in contrast with previous studies, our findings suggest that a higher mitotic activity may occur during the proliferative phase in myometrium and leiomyoma cells and that there is a significant decrease in the PCNA expression during the secretory phase, which further supports this hypothesis. This is the first study comparing PCNA protein expression in tissue extracts using Western blot analysis in addition to immunohistochemistry. We therefore believe that our results should be more reliable and may reveal more quantitative information compared with previous studies' immunohistochemical scoring system only. On the other hand, since our results are mostly descriptive, a functional and in vitro analysis of these proteins' function and regulation by menstrual cycle regulators such as sex steroids is needed.

In a recent study, many genes were compared in myometrium and leiomyoma using microarray analysis.[31] PCNA is one of the genes, found to be increased in

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leiomyoma at the mRNA level. Dixon et al[32] suggested that regardless of the menstrual phase, individual tumors in the same patient showed differential expression of proliferation markers. For example, examination of the tumors in 1 patient revealed PCNA labeling indices that ranged from less than 1% to 5.8% for different leiomyomas exposed to the same hormonal milieu in the same patient. These data suggest that factors other than the hormonal milieu are important in regulating the growth of uterine leiomyomas.[32] There is evidence to suggest that sex steroids are not the sole modulators of leiomyoma tumorigenesis and growth since steroid hormone levels in women with leiomyomas are similar to those in normal women.[33]

To our knowledge, this is the first study investigating PTEN expression in either the myometrium or leiomyoma. Our results show that leiomyoma samples express a decreased level of PTEN. Although no change was detected in the expression of PTEN in myometrial cells throughout the cycle, the paired analysis revealed a significant decrease in the PTEN level in leiomyoma only during the proliferative phase. Mutter et al[34] and Guzeloglu-Kayisli et al[35] have reported changes in the endometrial PTEN expression throughout the menstrual cycle. Guzeloglu-Kayisli et al[35] have also shown that estrogen increases PTEN phosphorylation while progesterone increases PTEN level in endometrial stromal cells, and they suggested that PTEN might be one of the signaling proteins that estrogen and progesterone are acting to affect endometrial cell proliferation and/or apoptosis. Thus, with our current findings, we may speculate that the decreased level of PTEN may also be involved in the regulation of increased cell proliferation of leiomyoma cells, which needs further in vitro functional analysis. Since PTEN blocks Akt phosphorylation (activation), we should expect to observe increased levels of Akt phosphorylation in leiomyoma compared with myometrium. Recent studies, which support this hypothesis, showed that the expression of Akt protein as well as its phosphorylation levels were higher in leiomyoma than in the corresponding myometrium.[36,37] Steroid receptor coactivators are also implied to play a role in the response of leiomyoma smooth muscle cells to estrogen, via estrogen receptor α. However, one recent study showed that changes in their levels do not appear to contribute to the increased sensitivity of these cells to estrogen.[38] This in turn may show that the nongenomic interaction of estrogen signaling in leiomyoma may be equally, if not more, important than its genomic signaling.

Our results show that the expression of FasL, PTEN, and PCNA differs in leiomyomas and in myometrium and suggest that cycle-dependent changes in the expression of these proteins may likely play a role in the myometrial physiology and may be involved in cell survival events (balance between proliferation and apoptosis). Changes in the expression levels of PCNA, FasL, and PTEN in leiomyoma suggest a role for antiapoptotic and proliferative proteins in the pathogenesis of leiomyoma. Furthermore, we may speculate that an increase in the FasL level in leiomyoma is likely to correspond to suppression of local immunity by inducing apoptosis of immune cells, which needs further studies. In this study, we obtained descriptive and semiquantitative results from in vivo tissues rather than establishing a direct proliferative or apoptotic index relationship. Therefore, further functional and in vitro experiments to analyze these indices are needed.

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