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報告
子宮肌瘤經過 GnRH-a 治療後與細胞生長、修補及凋亡有關之分子的變化

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計畫主持人：黃順賢
共同主持人：
計畫參與人員：

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Abstract
GnRH agonist (GnRH-a) therapy is known to shrink uterine leiomyoma,
previous observations that GnRH-a therapy fails to increase apoptosis in uterine leiomyomas. Uterine leiomyoma, a common pelvic tumor of reproductive age developing to a significant morbidity on occasion, is a smooth muscle tumor with such features as increased cellularity and cell hypertrophy (1). Its growth depends on ovarian steroid hormones and some growth factors. It has been demonstrated that monthly administration of a long-acting GnRH agonist (GnRH-a) in patients with uterine leiomyomas significantly reduces tumor size and serum estradiol levels (2, 3, 4). In addition, GnRH-a therapy decreases estrogen and progesterone receptors (5), trophic factors such as epidermal growth factor (6), IGF-I (7), and uterine blood flow (8), all of which are required for the growth of leiomyomas. Significant shrinkage in tumor size is especially observed within the first 2 months of GnRH-a administration, thus regarded useful in facilitating the removal of leiomyomas (2, 3, 4). However, the tumor shrinkage becomes less obvious even with continued treatment, and tumor growth is apt to recur after drug cessation (4).

The molecular mechanism by which GnRH-a acts on uterine leiomyomas has not been fully resolved. The effect of GnRH-a on reducing tumor volume can be mediated by the induction of cellular atrophy of leiomyoma cells (9) and/or the decrease of the tumor cell number (resulted from cell death induction and mitosis suppression) (10). Conflicting results exist on cell death induction in which researchers have reached no agreement on whether a concomitant increase of apoptosis rate could be found with GnRH-a therapy-induced tumor shrinkage (10, 11, 12, 13). Our previous work (13, 14) pointed out that GnRH-a-induced leiomyoma shrinkage was due at least in part to a mechanism involving DNA damage and cell cycle arrest indirectly through estrogen action on the tumor level. However, the DNA damage could be successfully repaired later on, and thus the rate of apoptosis was not increased.

The importance of apoptosis in physiology and pathology is now widely recognized. The family of cysteine proteases, known as the caspases, provides critical mediators for apoptosis. These caspases existing primarily in cells as inactive zymogens, called procaspases, are activated by proteolysis in a pattern similar to complement activation when the cell death signal is triggered. The cell death signal can be initiated by the direct ligation of death receptors such as Fas on the cell surface with its ligand, the former of which in turn passes the signal down to the activation of initiator caspases 8 or 10 (15). Activated caspase 8 cleaves Bcl-2 interacting domain proteins, and then the cleaved products work through Bcl-2 family members that lead to the activation of downstream caspases and ultimately cell death (16). Bcl-2, antiapoptotic itself, somehow blocks the pore or channel opening in the mitochondrial membrane (17).

Alternatively, irreparable damage on genome caused by mutagens, pharmaceuticals, or ionizing radiation makes cytochrome c released from mitochondria, which in turn activates caspase 9 by triggering the interaction of apoptosis-activating factor-1 with procaspase 9. The processed caspase 9 propagates the death signal by triggering downstream executioners—caspases 3, 6, and 7—until cell death (15, 16, 18). In addition, caspase 12 can be activated distinctly by stress signals from endoplasmic reticulum instead of signals from membrane or mitochondria (19). Fas (CD95/APO-1) and its ligand (FasL) are proteins as members of the TNF family. The Fas/FasL system is important in regulating immune function, developing organs, and conferring
immune privilege (20, 21, 22). Disturbance of this system has been linked with the pathogenesis of diseases such as autoimmune disease and cancer. Overexpression of FasL in cancers may evade immune surveillance by eliminating infiltrating lymphocytes (23). However, its role in the growth and development of benign tumors such as leiomyoma is not known. Further studies have demonstrated that Fas and FasL are not solely restricted to lymphoid or malignant cells (24). Fas and FasL are coexpressed in epithelia such as esophagus, prostate, lung, and uterus, in which their physiological functions are not known (25). Despite expressing Fas, many cells are resistant to Fas-mediated apoptosis. Even in the same tissue, coexistence of Fas-sensitive and Fas-resistant cells had been demonstrated (26).

GnRH-a therapy reportedly causes apoptosis in tumors of prostate, ovary, endometrium, and breast by increasing membranous FasL expression to attack Fas-positive cells within the tumors (27). Whether GnRH-a therapy induces apoptosis in uterine leiomyomas, however, remains to be determined. Furthermore, the alterations of Fas/FasL system and caspases in the aftermath of GnRH-a therapy are unknown so far. In this article, we have investigated the expression profiles of Fas and FasL, initiator caspases including Fas-associated caspases 8 and 10; caspase 9, dependent on the effect of cytochrome c released from the mitochondria; and the executioner caspases 3, 6, and 7 in leiomyoma cells, as well as control cells before and after GnRH-a therapy to figure out the drug effect on cell destiny.

**Materials and Methods**

**Tissue collection, preparation, and characterization**

The specimen pairs of leiomyomas and myometria were taken by myomectomy from patients treated approximately 2–3 wk previously with three doses of Leuplin Depot [leuprorelin acetate depot (LA), Takeda Chemical Industries, Ltd., Tokyo, Japan], a long-acting GnRH-a, as the LA-treated group in this study, or done by hysterectomy from premenopausal women receiving no exogenous hormonal or GnRH-a therapy during their follicular phase of menstrual cycle as the control group. The works of these investigations were conducted after obtaining informed consent from patients and approval from the institutional review board at National Cheng Kung University Hospital (Tainan, Taiwan). We used ultrasound to estimate the tumor sizes 1 d before surgery. For the LA-pretreated patients, additional scannings were performed before and during LA treatment. The details on the method of tumor size estimation and response after treatment were described previously (13). The representative samples were taken from distinct nondegenerating leiomyomas and a separate site at least 1 cm away from the tumor as normal myometrium. The pathology of the surgical specimens was confirmed by fellow pathologists.

**Tissue cell lysate preparation**

Frozen tissue was weighed, sliced in very thin layers, and thawed in ice-cold radioimmune precipitation assay buffer by 3 ml/g tissue. Tissue was further disrupted and homogenized by a dounce homogenizer. The working temperature was maintained at 4 C throughout all procedures in this section if a particular temperature condition is not mentioned. An appropriate amount of protease inhibitor stock (Calbiochem-Novabiochem Co., La Jolla, CA) was added to the crude homogenate to make a 1x working cocktail solution containing 500 µM 4-(2-aminoethyl) benzenesulfonylfluoride hydrochloride, 1 µg/ml aprotinin, 1 µM E-64 protease inhibitor, 500 µM EDTA, and 1 µM Leupeptin, and then incubated on ice for 30 min. The crude homogenate was transferred to microfuge tube and
centrifuged at 15,000 x g for 30 min at 4 C. The supernatant was removed and microfuged again. The latter supernatant was collected as total cell lysate. Protein concentration was determined by the Bio-Rad Laboratories, Inc. protein assay.

**Immunoblotting analysis**

Because the tissue lysate might contain a various proportion of extracellular protein, intracellular β-actin was used to ensure the even loading of protein samples on SDS-PAGE. The protein samples separated by SDS-PAGE were then transferred onto nitrocellulose membranes by a semi-dry blotting system. The membranes were blocked in 1x PBS containing 5% (wt/vol) skim milk at room temperature for 1 h, washed in a mixture of PBS and 0.05% Tween 20 (Tween-PBS; Sigma Chemical, St. Louis, MO), and then incubated overnight at room temperature with desired antibodies for 1 h. The specific antibodies used for particular protein detection were as follows: anti-FasL antibody (CD95L antisera Clone 33; Transduction Laboratories, Inc., Lexington, KY); anti-Fas antibody (G254–274; PharMingen, San Diego, CA); anti-caspases 3 and 7 antibodies and anti-Bcl-2 antibody (Transduction Laboratories, Inc. and Immunotechnology, Villepinte, France); and anti-caspases 6, 8, 9, and 10 antibodies (Upstate Biotechnology, Inc., Lake Placid, NY). After being washed in Tween-PBS, the membranes were incubated with 1000-fold diluted, biotinylated antimouse IgG antibody conjugated with horseradish peroxidase (Amersham Pharmacia Biotech, Piscataway, NJ) at room temperature for 1 h and then developed by the enhanced chemiluminescence system (Amersham). The intensity of each band in Western blot was then analyzed by a laser densitometer (PD-120, Molecular Dynamics, Inc.; Sunnyvale, CA). To obtain comparable data from different films, a relative intensity was used and defined as the ratio of band intensity of each lane to that of its corresponding β-actin.

**RNA isolation and RT-PCR**

Tissues from LA-treated and untreated patients were minced by cryosection. RNA was prepared by the RNaseasy Total RNA kit according to the manufacturer’s instruction (QIAGEN, Hilden, Germany). Total RNA was converted to cDNA using oligo-dT as a primer and Maloney murine leukemia virus reverse transcriptase (Promega Corp., Madison, WI). The mixture was incubated at 42 C for 1 h and then heated to 95 C for 2 min to denature the reverse transcriptase. The generated cDNA was subjected to 35 cycles of PCR amplification in a PerkinElmer Corp. (Wellesley, MA) amplification cycler. For PCR amplification, each 20 µl reaction mixture contained 1 µl cDNA solution, 500 pM primers, 0.2 mM dNTP, 1.5 mM MgCl₂, and 0.5 U Taq DNA polymerase. The condition of thermal cycling was as follows: denaturation at 94 C for 30 sec, annealing at 54 C for 1 min, and extension at 72 C for 2 min. Gene-specific primers for FasL, caspase 3, and β-actin were selected as follows: forward primers, FasL, CTGGAATGGGAAGACACC; caspase 3, 5'-ATACTCCTTCCATCAAATAG-3'; and β-actin, AGCGGGAAATCGTGCGTG; reverse primers, FasL, AGATTCCCTAAAAATGATCAGAGAGAG; caspase 3, 5'-ATACTCCTTCCATCAAATAG-3'; and β-actin, AGCGGGAAATCGTGCGTG; reverse primers, FasL, AGATTCCCTAAAATTGATAAGAG; caspase 3, 5'-AACATCACAACAAACCATAATC-3'; and β-actin, CAGGATCATAGTG GTGGTGCC. These primer sets for FasL, caspase 3, or β-actin genes produced amplicons in 338, 410, or 309 bp, respectively. The amplified RT-PCR
products were analyzed by electrophoresis through 1% agarose gels, visualized by ethidium bromide staining, and photographed under UV illumination.

**Immunohistochemistry**
The localization of Fas and its ligand was investigated by immunostaining using an IgG anti-Fas monoclonal antibody (Clone 13, Transduction Laboratories, Inc.) and an IgG anti-FasL polyclonal antibody (N-20, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Sections from formalin-fixed, paraffin-embedded archival tissues were dewaxed, processed to block endogenous peroxidase activity, permeabilized with proteinase K, and then immunostained by the ABC method (Dakopatts, Cambridgeshire, UK) in which anti-Fas antibody in a dilution of 1:200 and anti-Fas in 1:50 were used for overnight incubation, followed by incubation with a goat antirabbit IgG conjugated with horseradish peroxidase (DAKO Corp., Santa Barbara, CA). The immune complexes were visualized then by diaminobenzidine staining and hematoxylin counterstaining after extensive washing. Positive membrane staining on endothelial cells of the vessels within the leiomyomas served as positive control for FasL. The vascular smooth muscle cells (VSMC) of leiomyomas exhibiting Fas receptor in the cytoplasm (28) were positive control for Fas. At the same time, we performed immunohistochemical staining on another slide by missing first antibody as negative control.

**Statistics**
Data obtained from densitometry were expressed as mean ± SD. Statistical analysis was conducted using the paired t test, unpaired t test, or signed rank test as appropriate (Statistical Package for Social Sciences for Windows, version 7.5; SPSS, Inc., Chicago, IL). Statistical significance was established at P values of less than 0.05.

**Results**

**Clinical findings**
From January 1998 to June 2001, a total of 20 consecutive patients who had undergone myomectomy with prior LA treatment at the National Cheng Kung University Hospital (Tainan, Taiwan) were included. Twenty-four patients who had undergone myomectomy during the study period but received no prior LA treatment were selected as untreated patient controls. Ages ranged from 29–37 yr (mean, 33 yr) in the LA-treated patient group and from 32–38 yr (mean, 35 yr) in the control patient group. The volume of leiomyomas ranged from 33–444 ml before LA treatment and was decreased to 16–36 ml after treatment by a reduction rate from 41% to 97%. Thus, all of these patients were deemed as good responders according to our criteria (13). The volume of leiomyomas in LA-naive patients ranged from 14–502 ml. There was no significant difference between the two groups with respect to age, parity, and uterine size.

**The expression of Fas/FasL and caspases**
Fas, FasL, and all of the caspases investigated in their inactive form existed in both uterine myometria and leiomyomas. Figure 1 shows the representative Western blot results of FasL, Fas, and caspase 3 in the pairs of myometrium and leiomyoma from LA-treated and LA-untreated patients, accompanied with relative band intensities and their statistical data on these 20 LA-treated and 24 LA-untreated patients. All of the LA-untreated leiomyomas had up-regulated the expression of FasL as compared with their homologous myometria (P < 0.003; paired t test). A significant decrease in FasL expression was observed in the LA-treated myometria and LA-treated leiomyomas as compared with nontreated controls (P < 0.01 and 0.0001, respectively; unpaired t test). Besides, Fas expression was significantly
Leiomyoma has up-regulated the expression of caspase 3 as compared with the paired myometrium \((P < 0.0001; \text{paired } t \text{ test})\). Nevertheless, no active form of caspase 3, which appeared as a 20 kDa caspase 3 cleavage product, was detected. A significant decrease in caspase 3 expression was observed in both LA-treated myometria and LA-treated leiomyomas as compared with nontreated controls \((P < 0.0005 \text{ and } 0.0001, \text{respectively}; \text{unpaired } t \text{ test})\).

![Figure 1](image.png)

**Figure 1.** A, Representative FasL, Fas, and caspase 3 expressions in the pairs of leiomyomas (T) and myometria (N) from two patients without prior LA [LA(-)] and two patients with prior LA treatment [LA(+)]. Western blots showed that LA-untreated leiomyomas expressed more FasL and caspase 3 than homologous myometria or LA-pretreated leiomyomas. No cleavage product of caspase 3 was detected in all the conditions. \(\beta\)-actin was used to ensure the even loading of each specimen. B, FasL, Fas, and caspase 3 expressions in the pairs of leiomyomas and myometria from 20 LA-treated patients and 24 LA-untreated patients. FasL, Fas, and caspase 3 levels were measured by densitometry, and each column was presented in mean ± SD. Relative intensity was defined as the ratio of band intensity in each lane to the corresponding intensity of \(\beta\)-actin by densitometric measurement. *, A significant difference \((P < 0.05)\) in the comparison between LA-untreated leiomyomas and paired myometria using paired \(t\) test; **, a significant difference \((P < 0.05)\) in the comparison between LA-treated and LA-untreated myometria using unpaired \(t\) test; ***, a significant difference \((P < 0.05)\) in the comparison between LA-treated and untreated leiomyomas using unpaired \(t\) test.

![Figure 2](image.png)

**Figure 2** shows the representative expressions of caspases 6, 7, 8, 9, and 10 in the pairs of myometrium and leiomyoma from control patients as well as leiomyoma from LA-treated patients. In contrast to FasL and caspase 3, caspases 6, 7, 8, 9, and 10 did not possess any increased expressions in leiomyomas. LA treatment resulted in the decreased expressions of caspases 7, 9, and 10 \((P < 0.05; \text{unpaired } t \text{ test})\) but not caspases 6 and 8. However, we could not give any conclusion on whether LA-treated myometria also displayed any decreased expression of caspases 7, 9, and 10 because our myometria lysates collected from LA-treated patients were not adequate enough for sufficient repetition for the caspase items.
Representative caspases 6, 7, 8, 9, and 10 expressions in the pairs leiomyomas (T) and myometria (N) from three patients without prior LA therapy [LA(-)] and in leiomyomas from three LA-pretreated patients [LA (+)]. Compared with the myometria, leiomyomas did not have consistently elevated levels of these caspases examined. The depressive expressions of caspases 7, 9, and 10 were observed in LA-treated leiomyomas as compared with nontreated cases ($P < 0.05$; unpaired $t$ test). ß-actin was used as loading control.

**Semiquantitative RT-PCR of FasL and caspase 3**

A semiquantitative RT-PCR was applied to examine the RNA levels of FasL and caspase 3. The representative RT-PCR data show that the transcripts of FasL and caspase 3 genes in both LA-untreated myometrium and LA-untreated leiomyomas were in equal amounts, whereas those of sample pairs with LA treatment were decreased (Fig. 3). Taken together with the protein expressions of FasL and caspase 3 shown in Fig. 1, these results indicate that the overexpression of FasL and caspase 3 in untreated leiomyomas was up-regulated at the posttranscriptional level, whereas LA treatment suppressed the transcription of the genes in question.

**Immunolocalization of Fas and FasL**

To further investigate the factors that might affect the sensitivity of uterine smooth muscle cells to Fas-induced apoptosis, we assessed the cellular localization of Fas and FasL by immunohistochemical staining. We found that almost all of the FasL staining was confined to cytoplasmic or perinuclear regions of the cells (Fig. 4A). A granular pattern consistent with membranous staining at the periphery of the cells was detectable typically in endothelial cells but was not observed in leiomyoma cells. Besides, the Fas receptors were also found to localize in the cytoplasm, similar to what could be seen in VSMC (Fig. 4B).

**Figure 4.** Immunohistochemistry (x 400) of a LA-untreated leiomyoma revealed that both FasL (A) and Fas (B) were located within cytoplasm of the uterine smooth muscle cells. Leiomyoma cells, similar to VSMC in the same section, had cytoplasmically localized Fas receptor (B). Negative control studies were performed on another slide by...
missing first antibodies

**Bcl-2 expression**
To explore additional antiapoptotic mechanisms preventing cell death in leiomyomas, we studied the Bcl-2 expression. A representative Western blot result, which displayed Bcl-2 distributing in the specimen pairs of myometrium and leiomyoma from two LA-treated and two LA-untreated patients, is shown in Fig. 5A. The relative intensity and their statistical data on 20 LA-treated and 24 LA-untreated patients are shown in Fig. 5B. We found that leiomyomas possessed more Bcl-2 than their homologous myometria ($P < 0.002$; paired $t$ test) and the up-regulation of Bcl-2 in leiomyoma remained unchanged even under LA treatment ($P > 0.32$; unpaired $t$ test).

**Discussion**
The growth of uterine leiomyoma depends on ovarian hormones and some growth factors. GnRH-a is able to bring volume shrinkage to this tumor, although the molecular mechanisms of how GnRH-a acts on uterine smooth muscle cells remain to be elucidated. Other models suggest that GnRH-a therapy may activate apoptosis-associated pathways that involve the up-regulation of FasL (27). In uterine leiomyoma, conflicting results exist on whether GnRH-a therapy increases apoptosis of the cells (10, 11, 12, 13). In this article, we examined the apoptosis-associated pathways to see what the real destiny of leiomyomas would be under GnRH-a effect.

Our results demonstrated that Fas, FasL, and some caspase family members—notably caspases 3, 6, 7, 8, 9, and 10, all considered critical along the apoptotic pathways down to cell death—were constitutively expressed in uterine myometria. By comparison with their homologous myometria, leiomyomas showed an increased protein expression of FasL and caspase 3, both of which declined significantly under LA treatment in comparison with their LA-naive controls. The overexpression of FasL and caspase 3 in leiomyoma was due to tumorigenesis that increased the gene products at the level of posttranscription modulation, whereas LA decreased them at transcription level. The fluctuation of both FasL and caspase 3 expressions did not accompany any significant changes to the occurrence of apoptosis or caspase 3 cleavage activation. The absence of anticipated increase in caspases 6, 8, 9, and 10 in comparison between LA-untreated leiomyomas and paired myometria using paired $t$ test; $***$, no significant difference ($P > 0.05$) in the comparison between LA-treated and untreated leiomyomas using unpaired $t$ test.
leiomyomas might further confirm that neither Fas/FasL nor cytochrome c-related apoptosis pathways have been involved. Moreover, the decrease in Fas and in caspases 7, 9, and 10 and the consistently high level of Bcl-2 provided a further protection for leiomyoma cells from apoptosis under GnRH-a treatment. The result on Bcl-2 expression is in agreement with that of Matsuo et al. (29). In contrast, caspases 6 and 8 performed no level alterations in various conditions. Cellular localizations of Fas and FasL might be closely related to their biological function. The immunohistochemical study revealed that in the uterine smooth muscle cells Fas and FasL predominantly existed in the cytoplasm instead of on cell membrane as seen on activated lymphocytes or epithelial cells. Altogether, these findings may account for the relative resistance of leiomyomas to apoptosis and the limited effectiveness of GnRH-a therapy. They also support the hypothesis based on our previous report (13) and others (10) that GnRH-a therapy fails to induce apoptosis in uterine leiomyomas. The results also favor the findings in other models that Fas-induced apoptosis involves the complex and the coordinated interplay between proapoptotic and antiapoptotic proteins. Apoptosis through Fas/FasL system requires caspases 3, 8, and 9 for execution (30, 31). Despite the wide expression of Fas, different types of cells display a variety of sensitivity to Fas-induced apoptosis (32). In fact, Fas-mediated apoptosis requires sufficient density of Fas molecules on the cell surface, multimerization by the ligation of FasL, and the switching off of antiapoptotic programs in apoptosis-vulnerable cells. Only if all these requirements are met are cells susceptible to Fas-mediated apoptosis (33). It is why many kinds of cells remain resistant to Fas-induced apoptosis even expressing Fas, that cells perform the intracellular sequestration of Fas (28), the down-regulation of surface Fas expression, the expression of FasL decoy receptors (34), or the increased expression of antiapoptotic proteins such as Bcl-2 family members (17). Similar to uterine smooth muscle cells, human VSMC also have an intracellular pool of Fas and FasL. Some reports indicate that p53 activation transiently increases the surface Fas expression by facilitating the protein transportation from Golgi complex, resulting in apoptosis (28). However, VSMC exhibit a feature, marked heterogeneity to Fas-induced apoptosis, i.e. Fas-induced apoptosis is determined not only by surface Fas expression but also by the differential expression of specific death-signaling proteins below receptor level (26). Cells resistant to Fas-induced apoptosis, when compared with those vulnerable ones, exhibit a reduced expression of caspases 3 and 8 and an increased expression of antiapoptotic proteins. The biological significance of Fas and FasL coexpression in normal uterine smooth muscle cells and FasL up-regulation in the tumor cells remain to be determined. Uterus has been taken as an immune-privileged organ as for promising the success of pregnancy. It expresses both Fas and FasL (24). Constitutive FasL expression implies the maintenance of an immune privilege, and FasL overexpression in cancer is associated with the escape of immune surveillance (23). In the tissues capable of regeneration such as vagina (35) and esophagus (25), the coexpression of both Fas and FasL may play a physiological role in cell turnover. Furthermore, Fas is related to cell cycle progression in glioma (36). However, 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, for their cytosolic localizations preventing any probably
functional binding between Fas and FasL. Hormone-responsive tumors such as prostatic tumor, endometrial, ovarian, and breast cancer are sensitive to GnRH-a treatment. In addition to lowering growth factors induced by the treatment, GnRH-a might induce tumor growth arrest by stimulating apoptosis. Similar to the action of certain chemotherapeutic agents, GnRH-a increases Fas ligand expression on cell membrane, which is known to promote apoptosis through attacking Fas-bearing cells in tumors. The up-regulation of FasL was dependent on the presence of GnRH receptor (27). Uterine leiomyoma cells have been found bearing GnRH receptor (14). Interestingly, Wang et al. (37) recently reported that GnRH-a has some direct effects on the cultured leiomyoma cells, and GnRH-a treatment markedly increases apoptosis as well as the expression of Fas and FasL. To the contrary, our results indicate that GnRH-a treatment causes the down-regulated expression of FasL with no concomitant increase in apoptosis in uterine leiomyomas. The discrepancy probably lies in the fact that in our condition GnRH-a exerts its action more indirectly through estrogen suppression rather than directly on the tumor cells (14). Tissue ischemia resulted from the decreased uterine blood flow by GnRH-a treatment, and subsequent DNA damage and repair might account for the different outcome on protein expression between in vitro and in vivo experiments. It deserves further investigation that explores the molecules modulating apoptosis in uterine muscle cells to facilitate the management of this disease. Recently, the administration of somatostatin analog reportedly reduces the volume of uterus and leiomyoma in women with uterine leiomyoma (38). Studies using the combination of GnRH-a and somatostatin analog are also under investigation. Clearly, several questions still remain unanswered. First, the up-regulation of caspase 3 and FasL in uterine leiomyomas decreases significantly as tumors shrink after GnRH-a therapy, suggesting that the linking is unrelated to apoptosis but related to cell growth. The biological significance of caspase 3 and FasL in tumorigenesis deserves further investigation. Second, the precise locations of the intracellular Fas and FasL and the signaling mechanisms responsible for regulating the protein transportation to cell surface or extracellular secretion remain undefined. Third, the mechanism of differential activation of signaling pathways responsible for a different sensitivity of tumors to GnRH-a therapy remains to be determined. The better understanding of all these issues may facilitate GnRH-a therapy or relevant treatment against apoptosis-resistant tumors.

Acknowledgments

Footnotes

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Abbreviations: FasL, Fas ligand; GnRH-a, GnRH agonist; LA, Leuplin Depot; VSMC, vascular smooth muscle cells.

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