Increased progesterone receptor expression in uterine leiomyoma: correlation with age, number of leiomyomas, and clinical symptoms

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Objective: To investigate the possible correlation between progesterone receptor (PR) expression in uterine leiomyoma or adjacent myometrium and patient's age, size/number of leiomyomas, or clinical symptoms such as dysmenorrhea, acyclic pelvic pain, or menstrual and intermenstrual uterine bleeding.
Design: Cross-sectional study.
Setting: Referral center.
Patient(s): Sixty-two Chinese women undergoing elective hysterectomy for uterine leiomyomata.
Intervention(s): None.
Main Outcome Measure(s): Evaluation of PR-total and PR-B mRNA with real-time polymerase chain reaction; PR-A and PR-B proteins quantified by Western blot in leiomyoma tissue and myometrium; symptoms rated by the patients using visual analog scores.
Result(s): The PR-B mRNA and PR-A and PR-B proteins were more concentrated in leiomyomas than in matched myometrium. A direct correlation between PR-B mRNA levels in leiomyoma and age ($r = 0.347$) and number of tumors ($r = 0.295$) was found. Conversely, there was an inverse correlation between PR-B mRNA levels in leiomyoma and dysmenorrhea ($r = -0.260$) and intermenstrual bleeding ($r = -0.266$). Multiple regression analysis indicated that age ($\beta = 0.363$) and the number of myomas ($\beta = 0.296$) were independently associated with PR-B mRNA levels in leiomyoma tissue.
Conclusion(s): The levels of PR-B mRNA in leiomyoma tissue are directly associated with the number of tumors and inversely correlated with the intensity of intermenstrual bleeding and dysmenorrhea, suggesting that PR signaling may favor leiomyoma growth while attenuating clinical symptoms. This duality should be taken into account in the clinical management of patients with symptomatic uterine leiomyoma. (Fertil Steril® 2015;104:170–5. ©2015 by American Society for Reproductive Medicine.)
Key Words: Abnormal uterine bleeding, dysmenorrhea, myometrium, progesterone receptors, uterine leiomyoma

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Uterine leiomyomas (fibroids) are benign neoplasms constituted by islands of disordered smooth-muscle cells and large amounts of extracellular matrix (1, 2). Although their exact prevalence has not been verified in population-based studies, uterine leiomyomas have been found in over 70% of hysterectomy specimens from women in reproductive age (3). Common symptoms associated with these tumors include heavy menstrual bleeding, pelvic pain and discomfort, and subfertility (4).

The molecular mechanisms in the origins of uterine leiomyomas are unknown (5). Indirect evidence suggests that a single myometrial stem cell

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undergoes a specific gene mutation or a chromosomal rearrangement then starts dividing out of control and differentiates into a mature leiomyoma cell (1). The subsequent tumor development is highly dependent on sex steroid hormones that promote the proliferation and survival of leiomyoma cells through the inhibition of apoptotic pathways, the paracrine release of growth factors, and the accumulation of extracellular matrix (5, 6). Both estradiol and progesterone are critical for leiomyoma growth, and recent evidence shows that estradiol acts primarily by increasing cell responsiveness to progesterone (1).

The physiologic effects of progesterone are mediated by specific intracellular proteins: the progesterone receptors (PR), which belong to the nuclear receptor superfamily (7). The two main isoforms, PR-A (94 kDa) and PR-B (114 kDa), are products of a single gene, as a result of transcription from two alternative promoters or the translation from two alternative AUG codons in the full PR mRNA sequence (8, 9). Progesterone receptor B has a unique upstream segment and is a stronger activator for transcription of various promoters in response to progesterone, whereas PR-A is less active and may antagonize the transcriptional activity of PR-B at certain target genes (9).

Both PR isoforms have been shown in human myometrium and leiomyoma, without any significant variation during the menstrual cycle phases (10). However, in leiomyoma the PR expression is higher than in adjacent myometrium (10–13), explaining the evidence that progesterone is critical for tumor growth via cell proliferation and extracellular matrix accumulation (14, 15). These data offer the opportunity for the therapeutic use of progesterone antagonists or of selective PR modulators in an attempt to inhibit tumor growth; several clinical trials have shown the effectiveness of these drugs for leiomyoma treatment (16, 17).

Despite the pivotal role of progesterone in the pathophysiology of uterine leiomyomas, the relationship of local PR expression with the clinical manifestations of the disease has not been elucidated. Therefore, we investigated the possible correlation between PR expression in uterine leiomyoma and the number, size, and location of myomas and various clinical symptoms to evaluate the clinical significance of the molecular findings.

MATERIALS AND METHODS

Patients

The present cross-sectional study included a group of premenopausal Chinese women (n = 62) (age range: 23–54 years) with uterine leiomyomata who underwent hysterectomy at the First Affiliated Hospital of Soochow University, Suzhou, People's Republic of China, from June 2013 to May 2014. Histologic analysis confirmed the diagnosis of leiomyoma and excluded malignancy in all cases. Informed consent was obtained from each patient, and the study was approved by the local ethics committee.

The main clinical and demographic characteristics of the study participants are summarized in Table 1. All presurgical hormone treatments (oral contraceptive pills, progestins, levonorgestrel intrauterine system, or gonadotropin-releasing hormone analog) were interrupted at least 3 months before surgery, which was performed in the follicular phase of menstrual cycle. The uterine volume was calculated preoperatively by transvaginal ultrasound using the prolate ellipsoid formula (18). Dysmenorrhea and acyclic pelvic pain were measured by visual analog scale. Menstrual and intermenstrual bleeding were rated by the patient using a simple visual method (19) and were converted to an arbitrary scale ranging from 0 (absent) to 5 (heavy).

TABLE 1

Characteristics of the study participants (n = 62).

Characteristic	Median	Interquartile interval	Range
Age (y)	42	37–45	23–54
Dysmenorrhea (VAS)	3	2–4	1–7
Intermenstrual pelvic pain (VAS)	3	2–5	0–7
Menstrual bleeding	3	3–5	1–5
Intermenstrual bleeding	2	1–2	0–5
Largest leiomyoma diameter (mm)	65	50–80	12–130
Uterine volume (cm^3)	126	65–198	16–403
No. of leiomyomas	2	1–6	1–15
Predominant localization			
Subserous, n = 2			
Intramural, n = 55			
Submucous, n = 5			

Note: VAS = visual analog scale.

Tsigkou. PR, leiomyoma, clinical signs and symptoms. Fertil Steril 2015.

Sample Collection

Samples of leiomyoma (one for each patient) and normal myometrial tissue from the same patient were obtained in the operating room, snap frozen in liquid nitrogen, and stored at −80°C until processed. The frozen samples were finely diced and mixed, and weighed on a weight boat precooled over dry ice; 100 mg of each tissue sample were homogenized to a fine powder over a lake of liquid nitrogen using a precooled mortar and pestle.

RNA Extraction, Reverse Transcription, and Real-time Polymerase Chain Reaction

Total RNA was extracted from the homogenized tissue samples by the use of the Illustra RNAspin Mini RNA isolation kit (GE Healthcare). The extracted RNA was treated with DNAse to remove undesired genomic DNA contamination and was quantified by ultraviolet light absorption using a NanoDrop spectrophotometer. Complementary DNA synthesis from 1 μg of total RNA and real-time polymerase chain reaction (PCR) were performed sequentially using SuperScript III Platinum One-Step Quantitative RT-PCR System (Life Technologies/Invitrogen). The reaction conditions consisted of 50°C for 15 minutes, 95°C for 2 minutes, and 40 cycles of 15 seconds at 95°C and 30 seconds at 60°C. Melting curves showed a single amplicon and no primer dimers. All values of the target genes were normalized to the expression of the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sequences were PR (accession code