Spatial differences in biologic activity of large uterine leiomyomata

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Objective: To evaluate the growth pattern of the large uterine leiomyomata (ULM), we examined the spatial gene distributions, vessel density, proliferative activity, and hyaline degeneration.
Design: Tissue sections from three-dimensional large ULM, matched myometrium, and small ULM were collected and microarrayed. The spatial difference of the tumor activity was mapped in large ULM.
Setting: University clinical research laboratory.
Patient(s): Hysterectomy specimens from 7 patients with large (>10 cm) ULM and 3 patients with large (>10 cm) uterine leiomyosarcomas.
Intervention(s): Tissue microarray analysis by the immunohistochemistry.
Main Outcome Measure(s): Selected gene products, vessel density, and the percentage of hyaline degeneration were all scored in tissue cores/sections of large and small ULM against matched myometrium.
Result(s): We found that there was a spherical spatial difference of the tumor activities in large ULM. The tumor region next to the periphery, the most biologically active zone, demonstrated higher levels of gene expression, a higher density of vessels, a higher proliferative rate and a lower level of hyaline degeneration. The large ULM have higher levels of gene products (except for estrogen and progesterone receptors) than small ULM.
Conclusion(s): In comparison of the spatial patterns of the gene activity between the large ULM and the large uterine leiomyosarcoma, the large ULM illustrate a growth pattern of nutritional dependence. (Fertil Steril® 2006; 85:179–87. ©2006 by American Society for Reproductive Medicine.)

Key Words: Uterine leiomyoma, leiomyosarcoma, tissue microarray, immunohistochemistry, three dimensions, ER, PR, hypoxia

Uterine leiomyomata (ULM) are the most common neoplasms in women of the reproductive age, with a lifetime accumulating risk of 70% in white and 80% in black women (1). Although patients with large ULM account for a small proportion of ULM in the general population, they often present with unbearable symptoms and eventually seek surgical intervention. The annual hysterectomies in the United States due to large and symptomatic ULM number approximately 200,000 (2). Medical costs and patients' suffering associated with ULM are substantial (3).

ULM are benign smooth muscle tumors driven by sex steroid hormones. The size and tumor growth rate are associated with the presence of the sex steroid hormones; the tumor size, for instance, is reduced in postmenopause and with the use of GnRH agonist treatment. The large ULM, however, do have several growth features that appear to be independent of the sex steroid hormone status: 1) Most large ULM are found in women in the late, rather than early, reproductive age (4); 2) large ULM exhibit a faster growth rate than small ULM (5); 3) large ULM present a lower level of up-regulation of estrogen receptors (ER) and progesterone receptors (PR) than small ULM (6); and 4) patients with numerous ULM often have only one or a few large ULM (our clinical observation).

It is poorly understood why some large ULM continue to grow fast, despite their histologically presented disadvantages of high levels of hyaline degeneration, necrosis, and fibrosis, and other ULM remain small. For those large ULM treated by GnRH agonists, the hyaline degeneration and necroses are even broader than those of untreated ones (7). Once treatment is stopped, the tumors quickly resume their original sizes (8). Apparently, the large ULM continue to grow faster than small ULM (5), even in the presence of high degrees of these degenerative changes. We presume that there are spatial differences of the tumor activity in the large ULM that may contribute to this growth behavior. The highly active regions may be determined either by the local nutritional status or by the genetic alterations.

In this study, we explored the tumor growth behaviors by examining the spatial distributions of the ULM-associated gene expression, tumor proliferation rate, vessel density, and the levels of hyalinized degenerations in large leiomyomata. We found that the tumor region next to the peripheral zone is the area with higher levels of the gene expression, higher vessel densities, higher proliferation rates, and lower hyaline

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TABLE 1

Summary of seven cases with large leiomyomata and three cases with leiomyosarcomas.

Case no.	Age (yrs)	Race[a]	Size (cm)	Weight (g)	Phase[b]	No. of tumors	MM cores	Large ULM/LMS cores			S-ULM cores	Cytogenetics[c]
								X	Y	Z		
ULM1	34	B	10	1550	S	5	2	5	5	5	2	ND
ULM2	38	B	10	610	S	3	2	6	6	6	2	NM
ULM3	47	B	12	1950	P	12	2	6	6	6	2	NM
ULM4	45	W	11	1350	P	7	2	6	6	6	2	NM
ULM5	52	W	13	1850	I	4	2	6	6	6	2	del (7)(q21–q31)
ULM6	44	A	13	1300	P	8	2	6	6	6	2	NM
ULM7	46	W	10	1050	P	10	2	6	6	6	2	1+,add(1)(p10)
LMS1	55	W	14	1350	I	1	2	6	6	6	0	Complex
LMS2	53	O	12	1400	I	3	2	6	6	6	2	Complex
LMS3	63	O	17	946	I	2	2	6	6	6	2	Complex

Note: ULM = uterine leiomyoma; LMS = uterine leiomyosarcoma; MM = myometrium; S-ULM = small uterine leiomyoma.

[a] B = black; W = white; A = Asian American; O = other.

[b] S = luteal phase; P = follicular phase; I = inactive endometrium.

[c] ND = not determined; NM = normal karyotype; Complex = multiple numerical and structural anomalies.

Wei. Spatial differences in large leiomyomata. Fertil Steril 2006.

degeneration. The spatial difference is one of the key factors in determining growth behavior in large ULM.

MATERIALS AND METHODS

Patients and Specimens

Seven hysterectomies with large ULM (≥10 cm) were collected in fresh tissues. The patients' ages, tumor sizes, cycles, and ethnicities are summarized in Table 1. Tissues from normal matched myometrium, one large ULM (≥10 cm), and two small ULM (<2 cm) were collected from each patient, and all except one large ULM received cytogenetic analysis. Three hysterectomies with large uterine leiomyosarcomas (LMS; ≥10 cm) were also collected as part of this study (Table 1). This work was approved by our IRB committee.

Three-dimensional Tissue Preparation and Tissue Microarray (TMA)

Wide strips of tissue from each large leiomyoma were dissected in the three dimensional axes X, Y, and Z (because no specific tumor orientation was defined, the X, Y, and Z axes were randomly chosen). Each axis was further orderly divided into 6 zones of approximately 1 cm in width (ranging from zone 1 of the periphery to zone 6 of the center) (Fig. 1). From each case, one section from each zone of each axis, 2 sections from small leiomyomata (<2 cm), and 2 sections from matched myometrium were prepared.

Hematoxylin and eosin (H&E)–stained slides from each section (1.5 × 1.0 cm) were reviewed to: 1) calculate the percentage of hyaline degeneration; and 2) mark the cellular and viable areas for tissue microarray selection. Tissue cores (0.6 mm) from each tissue section were microarrayed into a TMA recipient block. A total of 220 tissue cores were collected for the study.

ULM-Associated Gene Products

A group of ULM-associated gene products were selected for the study based on data from gene transcription profiles and previous studies (6, 9). This group of genes are abnormally expressed in ULM and cover broad functions including: [1] sex steroid hormone receptors: ERα and PR-A; [2] other nuclear receptors: retinoid X receptor α (RXRα) (10, 11) and glucocorticoid receptor (GCR) (9, 12); [3] growth factors: epithelial growth factor receptor (EGFR) (9) and insulin-like growth factor 2 (IGF2) (12–20); [4] apoptosis: Bcl-2 (21); [5] angiogenesis: vascular endothelial growth factor (VEGF) (18); [6] ULM-specific markers: human mobility group gene (HMGA2) (15) and CD24 (9, 11–13, 22); and [7] HIF1, MIB1, and Factor VIII: used to evaluate the proliferative activity, vessel density, and stress of hypoxia. The conditions for immunostaining have been described previously (9).

Immunoscores and Statistical Analysis

Stained TMA slides were graded jointly by two pathologists using a visual semiquantitation method (optical density of the immunoreactivity). A one-score system for immunointensity (II) was used for the markers showing a diffuse