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Aromatase cytochrome P450 and estrogen and progesterone receptors in uterine sarcomas: correlation with clinical parameters

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Abstract

We examined the immunohistochemical expression of aromatase cytochrome P450 (P450arom), estrogen receptor (ER), progesterone receptor (PR), and Ki-67 in postoperative uterine sarcomas ($n = 31$) and the corresponding eutopic endometria ($n = 20$) to evaluate the relationships between the endocrine character of uterine sarcomas and the clinical features. In sarcoma tissues, P450arom was detected in 55% of cases, ER in 42%, PR in 42%, and Ki-67 in 90%. In eutopic endometria, P450arom was detected in 60% of cases, ER in 60%, and PR in 35%. There were correlations in the steroid-related proteins between the tumors and endometria ($P = 0.001$–0.026). The positivity of endometrial P450arom ($P = 0.04$) and ER ($P = 0.006$) was higher in surviving patients than dead patients regardless of the menstrual state. The results demonstrate correlation between the expression of P450arom, ER, and PR in tumors and eutopic endometria. Intense expression of the steroid-related proteins was associated with better survival.

Keywords: Aromatase cytochrome P450; Estrogen receptor; Progesterone receptor; Uterine sarcoma; Prognosis

1. Introduction

Uterine sarcomas are rare tumors of mesodermal origin accounting for 3% of uterine malignancies [1]. Uterine sarcomas are associated with poor prognosis because of their aggressiveness and low sensitivity to chemotherapy, radiation, and hormone therapy. As detected in gynecologic neoplasms, estrogen receptor (ER), and progesterone receptor (PR) have been demonstrated in uterine sarcomas [2–5]. While the use of steroids in the treatment of uterine sarcomas has been suggested [5,6], no relationship between the receptor status and the response to adjuvant hormonal therapy has been shown [2,4].

Studies have shown that the steroid receptor-positive neoplasms of both benign tumors such as endometriosis [7,8], adenomyosis [8], and leiomyomas [9,10], and malignant tumors such as endometrial cancer [11,12], ovarian cancer [13], and breast cancer [14,15], also contain aromatase cytochrome P450 (P450arom), the enzyme responsible for estrogen biosynthesis. The local expression of P450arom may increase the local estrogen concentration, which together with the circulating estrogen stimulates the tumor growth. A preliminary study has shown aromatase activity in uterine sarcomas [16], however, the expression of P450arom and the relationship to the steroid receptors have not been studied. To reconsider the endocrine character of uterine sarcomas, we examined the immunohistochemical expression of P450arom, ER, PR, and the cell proliferation-associated antigen Ki-67 in both uterine sarcoma tissues and the corresponding eutopic endometria. We analyzed the relationships between the status of the steroid-related proteins and the clinical features.

2. Materials and methods

2.1. Subjects

A total of 31 cases of uterine sarcomas that had been pathohistologically diagnosed using the removed uteri were selected from the files at Kyoto Prefectural University of Medicine ($n = 11$) and Akashi City Hospital ($n = 20$). Of the 31 cases, 22 were leiomyosarcomas (LMS), five were endometrial stromal sarcomas (ESS), and four were malignant mixed mullerian tumors (MMMT) (Table 1). The

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Table 1
Expression of P450arom, ER, PR, and Ki-67 in uterine sarcomas

Case no.	Age (years)	Menstrual status[a]	Clinical stage	Survival	Histology[b]	Tumor				Eutopic endometrium		
						P450arom	ER	PR	Ki-67	P450arom	ER	PR
1	40	Pre	I	Alive	LMS	+[c]	+	++	+	+	+	+
2	47	Pre	I	Alive	LMS	+	++	+++	+	++	+	−
3	48	Pre	I	Alive	LMS	+	−	−	+	+	+	++
4	21	Pre	I	Alive	LMS	+	+++	+++	+	++	+++	+++
5	42	Pre	I	Alive	LMS	−	−	−	+	+	+	+
6	52	Pre	I	Alive	LMS	−	−	−	+	−	−	−
7	35	Pre	I	Alive	LMS	+	−	−	+	+	++	−
8	39	Pre	I	Alive	LMS	+	−	+	−	+	+	+
9	48	Pre	I	Alive	LMS	−	+	−	−	−	+	−
10	49	Pre	I	Alive	ESS	−	−	++	+	−	++	+
11	49	Pre	I	Alive	ESS	−	−	−	+	++	−	−
12	55	Post	I	Alive	LMS	+	−	++	+	++	+	−
13	61	Post	I	Alive	LMS	−	−	−	++	−	−	−
14	68	Post	II	Alive	MMMT	+	+	++	+	+	+++	++
15	51	Pre	III	Alive	LMS	+	−	+++	+	++	++	−
16	49	Pre	I	Alive	LMS	+	−	−	+	N.A.		
17	23	Pre	I	Alive	ESS	−	+	+++	+	N.A.		
18	60	Post	II	Alive	ESS	−	+	+	+	N.A.		
19	81	Post	III	Alive	LMS	−	+	−	++	N.A.		
20	71	Post	III	Alive	MMMT	+	+	−	++	N.A.		
21	59	Post	IV	Alive	LMS	−	−	−	+	N.A.		
22	44	Pre	I	Dead	LMS	−	+	++	−	−	−	−
23	52	Pre	I	Dead	LMS	+	+	++	+	+	−	−
24	57	Post	III	Dead	LMS	+	−	−	++	−	−	−
25	42	Pre	IV	Dead	ESS	−	−	−	+	−	−	−
26	46	Pre	IV	Dead	MMMT	+	−	−	+	−	−	−
27	49	Pre	III	Dead	LMS	+	−	−	++	N.A.		
28	47	Pre	IV	Dead	LMS	−	−	−	++	N.A.		
29	53	Post	IV	Dead	LMS	+	+	−	+	N.A.		
30	59	Post	IV	Dead	LMS	−	−	−	+++	N.A.		
31	51	Post	IV	Dead	MMMT	−	+	+	+	N.A.		

N.A., not available.
[a] Pre, premenopause; post, postmenopause.
[b] LMS, leiomyosarcoma; ESS, endometrial stromal sarcoma; MMMT, malignant mullerian tumor.
[c] The immunostaining intensity was classified into four grades: negative, weak, moderate, and strong, according to the criteria described in Section 2.

clinical stages at the operation were recorded according to the International Federation of Gynecologists and Obstetricians (FIGO) staging system for endometrial carcinomas. All of the patients had had no other endocrine disease. The tissues had been fixed with 10% phosphate-buffered paraformaldehyde and embedded in paraffin. In each case, 1–3 tissue blocks prepared from different portions of the tumors were subjected to immunohistochemical staining. In 20 of the 31 cases, the eutopic endometrial tissues were also available for evaluation of the histologic and immunohistochemical staining.

2.2. Immunohistochemistry

Immunohistochemical staining was performed as previously described [17]. Briefly, the tissue blocks were cut into 4 μm sections. The sections were deparaffinized, autoclaved at 121 °C for 20 min in 0.01 M citrate buffer (pH 6.0), treated for 5 min with 3% H_2O_2 to block the endogenous peroxidase, and washed three times with 0.05 M Tris–HCl buffer (pH 7.6) containing 0.05% Tween 20 (wash buffer). Autoclaving was omitted for the P450arom immunostaining. The sections were incubated for 10 min at room temperature with Dako Protein Block Serum-Free (Dako, Carpinteria, CA), and then for 24 h at 4 °C with a polyclonal antiserum against P450arom (PAb R-8-2, 1:1000) [18] or monoclonal antibodies (5 μg/ml, Dako) against ER, PR or Ki-67. The sections were washed three times with wash buffer, and incubated for 30 min at room temperature with biotinylated anti-rabbit or anti-mouse immunoglobulins (Dako). The sections were then washed three times with wash buffer, and incubated with streptavidin-conjugated horseradish peroxidase (Dako). The sections were colored with 3,3-diaminobenzidine hydrochloride solution, and counterstained with hematoxylin after three further washes in buffer.

For positive controls, chorionic tissues from patients undergoing elective termination of pregnancy between 6 and 9 weeks gestation for reasons other than gynecological ones were used. Negative controls for P450arom were incubated with the same dilution of nonimmunized rabbit serum or PAb R-8-2 that had been pretreated with immunopurified human placental P450arom (500 μg P450arom per 1 ml

diluted PAb R-8-2) to block the active site. Negative controls for the other antigens were incubated with the same dilution of nonimmunized mouse IgG.

For the immunostaining of tumor tissues, the staining intensity was classified into four grades: −, no tumor cells stained; +, less than 25% of the tumor cells stained; ++, 25–50% of the tumor cells stained; and +++, more than 50% of the tumor cells stained. For the immunostaining of eutopic endometrium, the staining intensity was graded using a semiquantitative index known as an *H*-score using the following equation: $H-\text{score} = \sum Pi$, where i is the intensity of staining with a value of 0, 1, 2 or 3 (negative, weak, moderate, or strong, respectively) and P is the percentage of stained cells for each given i (from 0 to 100%) [19]. The score was classified into four grades: −, *H*-score <20; +, between 21 and 100; ++, between 101 and 200; and +++, between 201 and 300. Approximately 500 cells were observed on the three slides that had been prepared from three different portions of each tissue, and the mean of two scores calculated by two independent observers was used for determination of the staining intensity.

2.3. Statistics

The relationships between the parameters were analyzed with Mann–Whitney's *U*-test, Spearman's correlation coefficient by rank test, and/or one factor ANOVA. A P value of <0.05 was considered significant.

3. Results

In the LMS tissues, P450arom was stained diffusely in the cytoplasm of tumor cells (Fig. 1A), although some cases displayed negative staining (Fig. 1B). There were no remarkable differences in the staining pattern among the histologic types. ER (Fig. 1C) and PR (Fig. 1D) were stained in the nuclei of tumor cells, almost focally except for two cases with diffuse immunostaining. In ESS, intense immunostaining was observed in areas with high vascularity. In MMMT, more intense immunostaining was observed in the cancerous cells than in the sarcoma cells. There was no remarkable difference between the localization of P450arom and the steroid receptors, although the localizations were not always the same. In cases where both ER and PR were expressed, they were immunolocalized in the same areas of the tumor. Ki-67 was stained diffusely in the nuclei of tumor cells (Fig. 1E).

In the eutopic endometrium of a uterus bearing a sarcoma, P450arom was immunostained exclusively in the cytoplasm of glandular epithelial cells and faintly in the stroma (Fig. 1F), although some cases displayed negative staining (Fig. 1G). ER (Fig. 1H) and PR (Fig. 1I) were immunostained in the nuclei of both epithelia and stroma, being more intense in the epithelia.

Of the 31 uterine sarcoma tissues, P450arom was immunohistochemically detected in 17 cases (55%; 13 of 22 LMS, 1 of 5 ESS, 3 of 4 MMMT). ER was detected in 13 cases (42%; 8 LMS, 2 ESS, 3 MMMT) and PR was detected in 13 cases (42%; 8 LMS, 3 ESS, 2 MMMT). Ki-67 was positive in 28 tumors (90%) (Table 1). Of the 20 cases of eutopic endometrial tissues, P450arom was detected in 12 cases (60%), ER in 12 cases (60%), and PR in seven cases (35%) (Table 1).

The relationships between the clinical and immunohistochemical parameters were analyzed statistically (Table 2). The clinical stage was higher in the dead patients than in the surviving patients ($P = 0.001$ by Mann–Whitney's

Table 2
Relationships among clinical parameters and immunohistochemical staining intensity of P450arom, ER, PR, and Ki-67 in the uterine sarcoma tissues and corresponding eutopic endometria

	Survival	Stage	Tumor			Endometrium	
			P450arom	ER	PR	P450arom	ER
Survival	–	–	–	–	–	–	–
Stage	0.001[a]	–	–	–	–	–	–
Menstrual state	NS	0.009[a]	–	–	–	–	–
Tumor							
P450arom	NS	NS	–	–	–	–	–
ER	NS	NS	NS	–	–	–	–
PR	NS	NS	NS	rs = 0.41, 0.004[b]	–	–	–
Ki-67	NS	0.005[c]	NS	NS	NS	–	–
Endometrium							
P450arom	0.04[a]	NS	rs = 0.74, 0.001[b]	NS	rs = 0.63, 0.009[b]	–	–
ER	0.006[a]	NS	NS	rs = 0.49, 0.026[b]	rs = 0.61, 0.01[b]	rs = 0.52, 0.03[b]	–
PR	NS	NS	NS	rs = 0.59, 0.006[b]	NS	NS	rs = 0.67, 0.007[b]

Values represent P values, rs, correlation coefficient. NS, not significant. (–) Values are listed in the other columns.
[a] Mann–Whitney's *U*-test.
[b] Spearman's correlation coefficient by rank test.
[c] One factor ANOVA.

U-test), and higher in the postmenopausal patients than in the premenopausal patients ($P = 0.009$ by Mann–Whitney's *U*-test). As the clinical stage progressed, the immunohistochemical intensity of Ki-67 in the tumor increased ($P = 0.005$ by one factor ANOVA). However, there were no significant relationships between the histologic types and the other parameters.

There were correlations between the immunostaining intensities of ER and PR in both the tumors (rs = 0.41, $P = 0.004$ by Spearman's correlation coefficient by rank test) (Fig. 2A) and the eutopic endometria (rs = 0.67, $P = 0.007$ by Spearman's correlation coefficient by rank test) (Fig. 2B). There was a correlation between P450arom and ER in the endometrium (rs = 0.52, $P =$

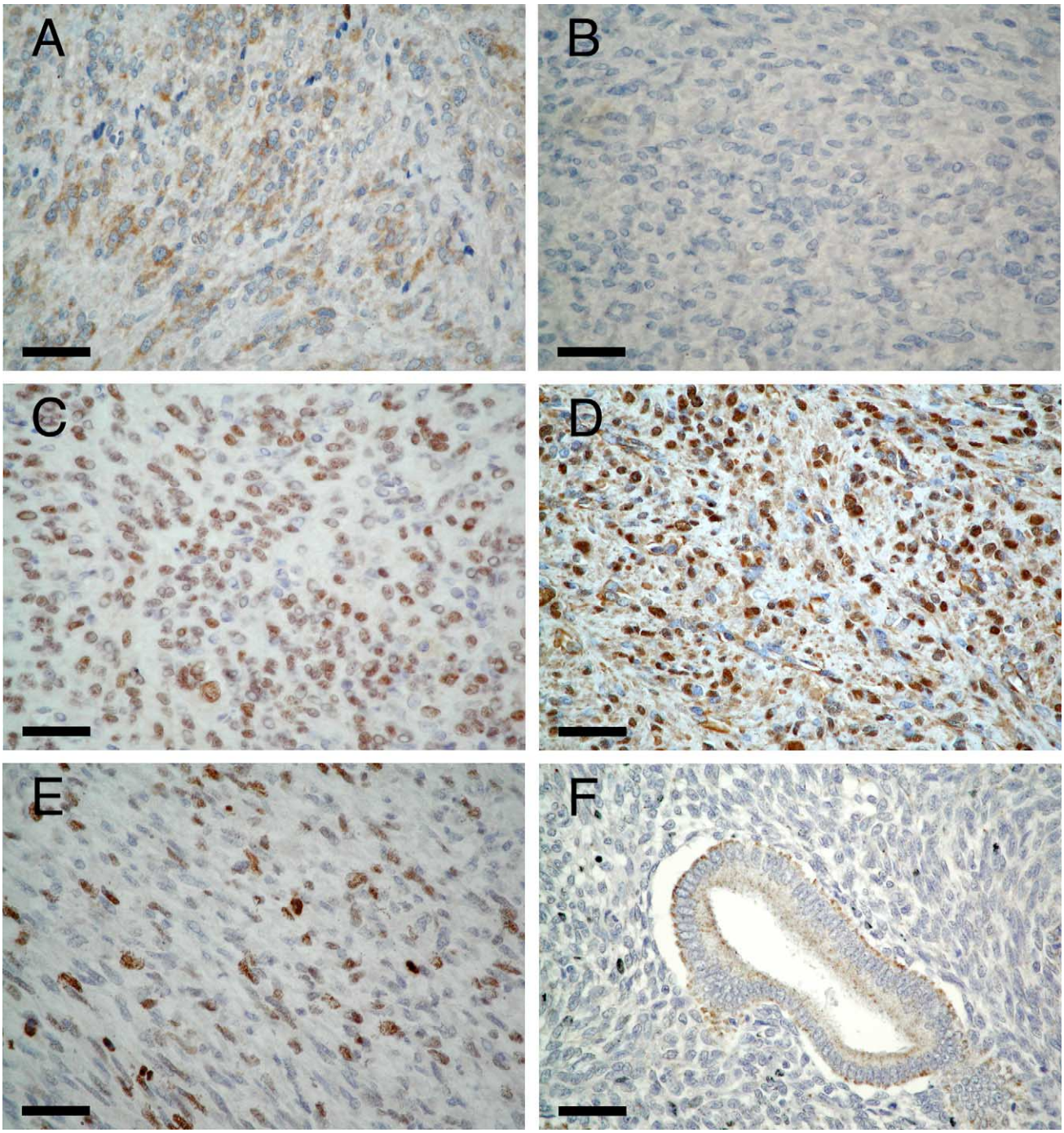

Fig. 1. Representative immunohistochemical staining for (A and F) aromatase cytochrome P450 (P450arom), (C and H) estrogen receptors, (D and I) progesterone receptors, and (E) Ki-67, (A–E) in the tumor and (F–I) the corresponding endometrium in leiomyosarcoma tissues. Negative P450arom staining in a (B) tumor and (G) eutopic endometrium. Original magnification, 300×. Bar = 20 μm.

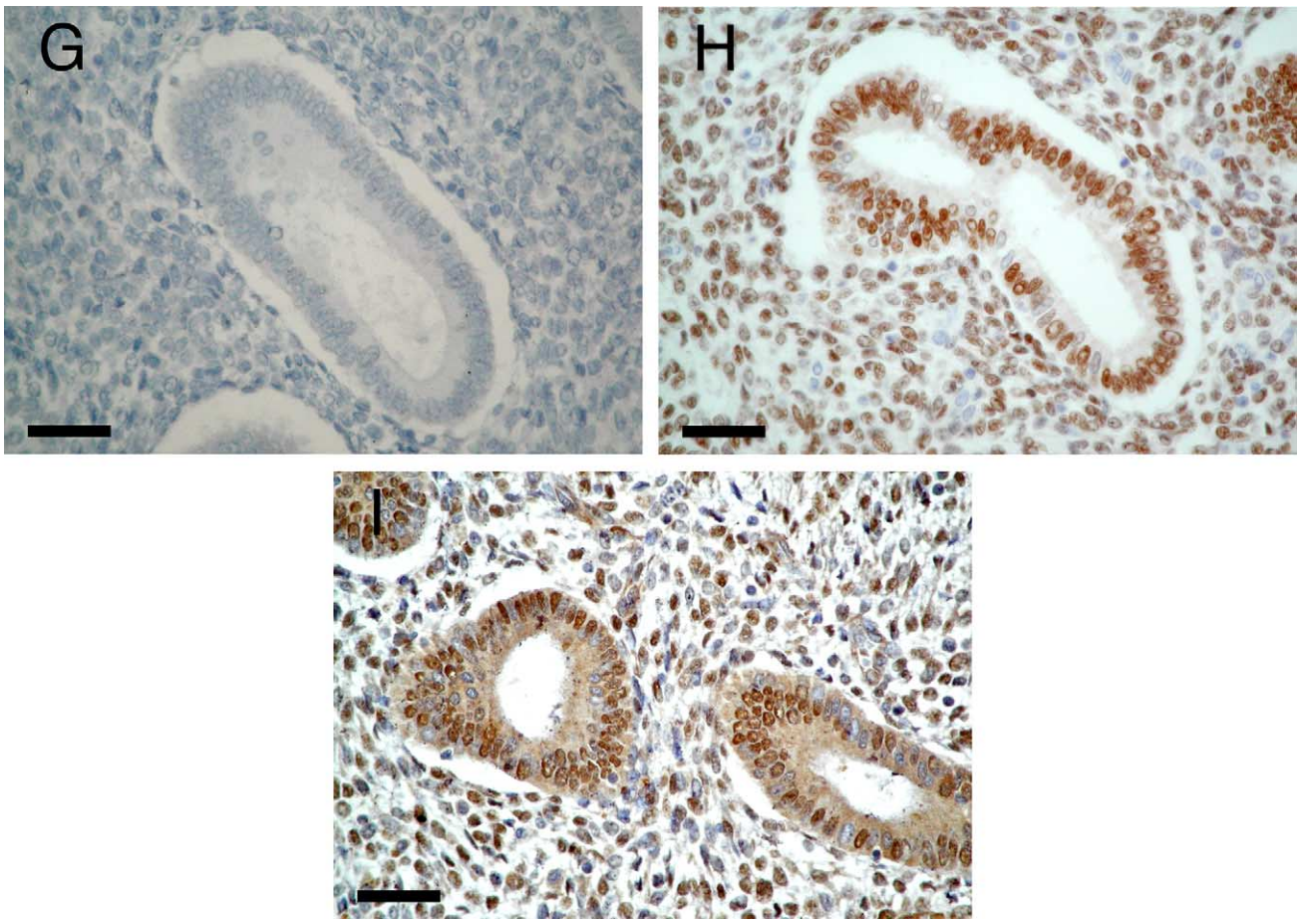

Fig. 1. (*Continued*).

0.03 by Spearman's correlation coefficient by rank test) (Table 2).

There were close relationships for the steroid-related parameters between the tumors and the eutopic endometria. There was a correlation between P450arom in the tumor and endometrium (rs = 0.74, $P = 0.001$ by Spearman's correlation coefficient by rank test) (Fig. 3), between the tumor ER and the endometrial ER (rs = 0.49, $P = 0.026$ by Spearman's correlation coefficient by rank test), between the tumor ER and the endometrial PR (rs = 0.59, $P = 0.006$

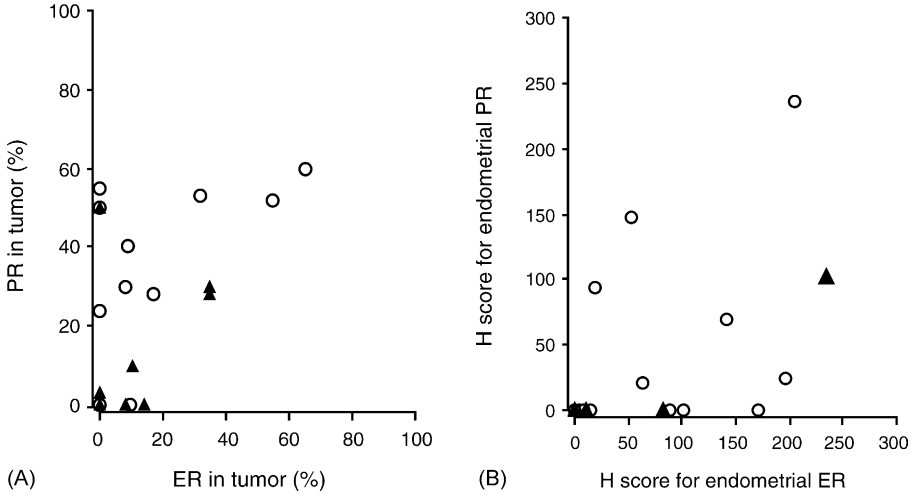

Fig. 2. Positive correlation between the immunostaining intensities of ER and PR in both the (A) tumors and the (B) eutopic endometria. (A) rs = 0.41, $P = 0.004$ and (B) rs = 0.67, $P = 0.007$ by Spearman's correlation coefficient by rank test. (○), Premenopausal patients; (▲), postmenopausal patients.

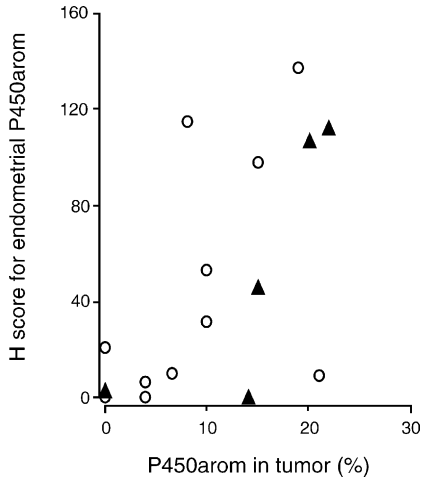

Fig. 3. Positive correlation between the immunostaining intensities of P450arom in the tumors and the eutopic endometria. rs = 0.74, $P = 0.001$ by Spearman's correlation coefficient by rank test. (○), Premenopausal patients; (▲), postmenopausal patients.

Table 3
The positivity of endometrial P450arom and ER was associated with better survival

	H-score				*P*[a]
	−	+	++	+++	
Endometrial P450arom					
Alive	4 (1)	6 (1)	5 (1)	0	
Dead	4 (1)	1	0	0	0.04
Endometrial ER					
Alive	3 (1)	7 (1)	3	2 (1)	
Dead	5 (1)	0	0	0	0.005

Numbers in the parentheses are postmenopausal patients.
[a] By Mann–Whitney's *U*-test.

by Spearman's correlation coefficient by rank test), between the tumor PR and the endometrial P450arom (rs = 0.63, $P = 0.009$ by Spearman's correlation coefficient by rank test), and between the tumor PR and the endometrial ER (rs = 0.61, $P = 0.01$ by Spearman's correlation coefficient by rank test) (Table 2).

Furthermore, tumor PR was negative in seven cases (70%) of dead patients. In all five cases of dead patients whose endometria were available for immunostaining, both endometrial ER and PR were negative, and in four cases (80%), except for one case, endometrial P450arom was negative. The immunohistochemical intensity of endometrial P450arom ($P = 0.04$ by Mann–Whitney's *U*-test) and ER ($P = 0.006$ by Mann–Whitney's *U*-test) was greater in the surviving patients than in the dead patients regardless of the menstrual state (Table 3).

4. Discussion

While the presence of steroid receptors in uterine sarcomas has been reported [2–5], the association between the growth of uterine sarcomas and sex steroids is not as well understood as it is in endometrial cancer and breast cancer. In the present study, ER and PR were detected in the uterine sarcoma tissues and the immunostaining intensity of both receptors was correlated, consistent with previous findings that there was a correlation between the levels of ER and PR determined by binding assays [2,3]. The expressions of ER and PR have been demonstrated in estrogen-dependent benign and malignant uterine tumors, ovarian cancer, and breast cancer. Interestingly, such tumors also express P450arom [7–16], which may increase the local estrogen concentration and stimulate the tumor growth. To the best of our knowledge, this is the first report to demonstrate the expression of P450arom in uterine sarcomas. Furthermore, this is the first study to examine the steroid-related parameters in the eutopic endometrium of patients with uterine sarcomas. The present study showed that P450arom, ER and/or PR were expressed in the eutopic endometrium of 50% of postmenopausal patients with sarcomas. While P450arom is not expressed in the eutopic endometrium of disease-free women, it is expressed in the eutopic endometrium of patients with endometriosis, adenomyosis and/or leiomyomas [8]. However, P450arom ceases to be expressed after menopause [17]. ER and PR also become faint in the endometrium of postmenopausal women who are disease-free or who have a benign disease. P450arom is the product of the *CYP19* gene, which is located on chromosome 15q21.1 with 10 exons. The tissue-specific gene expression of P450arom is regulated by multiple untranslated exon I [20]. Among the steroid-related parameters of P450arom, ER, and PR, there were correlations between many pairs of the parameters in the tumor and in the endometrium. Furthermore, the clinical stage was significantly higher in the cases of postmenopausal women than in the cases of premenopausal women. The positivity of endometrial P450arom and ER was significantly higher in the surviving patients than in the dead patients regardless of the menstrual state.

Taken together, it is suggested that the eutopic endometrium reflects the endocrine environment in the tumor tissue. It is also suggested that the intense expression of steroid-related proteins is associated with better survival. The present findings will be applicable to the clinical diagnosis of uterine sarcomas in postmenopausal women. For example, if P450arom, ER and/or PR are expressed in a biopsy specimen of the eutopic endometrium, a higher chance of sarcoma should be suspected.

While the present study showed no correlation between the expression of steroid receptors and the histologic types of sarcomas due to the limited number of cases, Navarro et al. [5] have suggested that ESS contains more abundant steroid receptors than the other two histologic types and that antiestrogenic drugs may be considered for the treatment of ER- and PR-positive ESS. Among actual cases, medroxyprogesterone acetate (MPA) was effective in the treatment of a case with multiple metastasizing LMS with the expression of ER and PR [6], whereas no relationship has

been shown between the receptor status and the response to adjuvant hormonal therapy [2,4]. However, one should be careful in evaluating the latter report because MPA as well as tamoxifen, which acts as an estrogen agonist, were used. Increased incidences of both uterine endometrial adenocarcinomas and sarcomas have been pointed out in women with breast cancer who are taking tamoxifen [21]. As demonstrated in the present study, uterine sarcomas exert in situ estrogen production and steroid receptor expression similar to estrogen-dependent benign uterine tumors such as leiomyomas. However, unlike leiomyomas, the expression of steroid receptors is less intense [22,23] and such an estrogenic environment may not continue to stimulate the growth of sarcomas but may regulate it. It is therefore possible that some cases may respond poorly to the hormonal therapy even if they contain ER and PR, and that some cases may actually worsen after the administration of hormones such as tamoxifen.

In conclusion, P450arom, ER, and PR were expressed in the tumor tissues and corresponding eutopic endometria of uterine sarcomas. High grades of correlations were found between the expression of the steroid-related proteins in the tumors and the eutopic endometria. Intense expression of steroid-related proteins was associated with better survival. Further investigation concerning the steroid metabolism and responsiveness needs to be performed. Examination of P450arom, ER, and PR in the tumor tissues and eutopic endometria may be useful in predicting the response to hormones and the prognosis in each case of uterine sarcoma.

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