

The Role of Steroid Sulfatase as a Prognostic Factor in Patients with Endometrial Cancer

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Purpose: The aim of the study was to determine steroid sulfatase (STS) expression in endometrial cancer patients and its correlation with disease prognosis.

Materials and Methods: We conducted a retrospective study in 59 patients who underwent surgery with histologically confirmed endometrial cancer from January 2000 to December 2011 at Hanyang University Hospital. Immuno-histochemical staining of STS was performed using rabbit polyclonal anti-STS antibody.

Results: Sixteen of the 59 patients (27.1%) were positive for STS expression. Disease free survival (DFS) was 129.83±8.67 [95% confidence interval (CI): 112.84–146.82] months in the STS positive group (group A) and 111.06±7.17 (95% CI: 97.01–125.10) months in the STS negative group (group B) ($p=0.92$). Overall survival (OS) was 129.01±9.38 (95% CI: 110.63–147.38) months and 111.16±7.10 (95% CI: 97.24–125.07) months for the groups A and B, respectively ($p=0.45$). Univariate analysis revealed that FIGO stage and adjuvant therapy are significantly associated with DFS and OS. However, in multivariate analysis, FIGO stage and adjuvant therapy did not show any statistical significance with DFS and OS. STS was also not significantly associated with DFS and OS in univariate and multivariate analysis.

Conclusion: STS expression was not significantly associated with DFS and OS, despite positive STS expression in 27% of endometrial cancer patients. Therefore, the role of STS as a prognostic factor in patients with endometrial cancer remains unclear and requires further research.

Key Words: Steroid sulfatase, endometrial cancer, prognostic factor

INTRODUCTION

Estrogen and progesterone production is essential for normal proliferation of the endometrium. Previous studies have shown

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that continuous and excessive exposure to estrogen increases the risk of endometrial cancer.¹⁻⁴ However, there is a paradoxical increase of estrogen-dependent endometrial cancer in postmenopausal women who have stopped producing estrogen in the ovaries. Hence, intrinsic synthesis and metabolism of estrogen plays an important role in the prevalence and progression of endometrial cancer.⁵ Inhibition of sex hormones in patients with endometrial cancer is thus an important therapeutic goal to reduce levels of biologically significant estrogen and achieve better prognosis.^{6,7}

Regulation of estrogen synthesis and action in the treatment of hormone-dependent breast cancer has been successful in the past.⁸ Tamoxifen, the estrogen receptor (ER) blocker has been standard treatment until now, and aromatase inhibitors (AIs) have been used as a first line therapy in metastatic and hormone-dependent breast cancer in postmenopausal women. AIs are also widely used currently, as first line therapy in

early ER positive breast cancer in postmenopausal women. Unfortunately, despite advances in therapeutic strategies, the majority of breast cancer patients who undergo these treatments have progressive disease after a few years of a complete response.

Androgen receptor (AR) is expressed in >80% of ER positive postmenopausal breast cancer women. However, AR is also expressed in ER negative patients. Androstenediol (Adiol) can bind to ER, despite being an androgen, and results in an increase in the number of ER positive breast cancer cells. Although Adiol has a weak affinity to ER, high concentrations of Adiol of >100 fold can have the same effect as estradiol (E2).⁹ Clinical treatment with AIs inhibits the synthesis of E2 by >99%, but simultaneously sensitizes to very E2 low concentrations. Adiol-induced low E2 concentration potentially affects the progression of breast cancer.⁸ The androgen-induced activation of estrogen through AR in ER-negative breast cancer patients is via steroid sulfatase (STS). Therefore, inhibition of STS can reduce estrogen synthesis by Adiol.

Most STS inhibitor clinical studies in breast cancer patients are in the clinical trial phase, and an ongoing study on hormone-dependent endometrial cancer that is similar to breast cancer is in the animal study phase. To the best of our knowledge, there is no study on STS with human endometrial cancer tissue. Therefore, we investigated STS expression in human endometrial cancer tissue and determined its correlation with prognosis in patients with or without expression of STS.

MATERIALS AND METHODS

Patient selection

We conducted a retrospective study in 59 patients who underwent surgery for endometrial cancer from January 2000 to December 2011 at Hanyang University Hospital. STS expression was confirmed through immuno-histochemical staining of sections from paraffin-embedded endometrial cancer tissue. We excluded patients who were diagnosed as recurrent endometrial cancer, transferred from another hospital after hysterectomy, and were lost to follow up after surgery. We evaluated patient characteristics, including age, parity, types of surgery, serum levels of CA125 and 19-9 before and after surgery, the International Federation of Gynecology and Obstetrics (FIGO) stage, histologic type and grade, and adjuvant therapies, such as chemotherapy, radiation therapy, and concurrent chemoradiation therapy (CCRT). In survival analysis, last day of treatment and follow up, day of recurrence, and day of death were investigated. The study was approved by the Institutional Review Board (Study approval No.: HYUH 2012-R-03).

Patient follow up

Patients were followed up by physical examination and for tumor markers (CA125/19-9) every 3 months for 2 years, and ev-

ery 6 months for the next 3 years. Chest X-ray, abdomino-pelvic computed tomography, and 18F-fluoro-D-glucose positron emission tomography-computed tomography (18FDG PET-CT) were performed annually. Recurrence of disease was defined as presence of tumor histologically or radiologically. Any suspicious lesion on CT scan was followed-up with CT scans every 3 months until recurrence was confirmed clinically. In cases of recurrence, 18FDG PET-CT was performed to locate other sites of recurrence, and all sites of recurrence were documented after 2006.

Surgery and adjuvant treatment

We performed total hysterectomy with bilateral salpingo-oophorectomy without lymphadenectomy in cases where the tumor mass was confined to the endometrium and there was no significant lymphadenopathy. Para-aortic lymphadenectomy was performed in cases where the tumor mass was ≥ 2 cm in size, or there were more than 1/2 invasions of the myometrium or positive pelvic lymphadenopathy in frozen sections.

We did not perform adjuvant treatment in patients with stage IA and grade 1, 2, or vaginal brachytherapy in patients with stage IA and grade 3, or stage IB. Patients with stage II received external pelvic radiotherapy and chemotherapy, and extended radiotherapy was given in patients with stage III or stage IV.

Construction of tissue microarray

The most morphologically representative and non-necrotic area was carefully selected and marked on the hematoxylin-eosin stained slide. Two tissue cores (2 mm in diameter) were sampled from each paraffin block and assembled into a recipient paraffin block using a tissue microarray instrument (AccuMax array, ISU Abix, Seoul, Korea).

Immunohistochemical staining

The 4- μ m-thick tissue sections were cut from the tissue microarray blocks and deparaffinized with xylene and rehydrated with graded alcohol. Antigen retrieval was performed by microwaving the samples for 12 min in preheated 10 mM sodium citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with peroxidase blocking solution (DAKO, Carpinteria, CA, USA) for 10 min. The rabbit polyclonal anti-STS antibody (ab62219, Abcam, Cambridge, MA, USA) was diluted 1:100 and incubated at 4°C for 16 h. The sections were then incubated with a peroxidase labeled anti-mouse/rabbit secondary antibody for 30 min (DAKO, Carpinteria, CA, USA). The samples were developed with DAB substrate (DAKO, Carpinteria, CA, USA) for 2 min and counterstained with Mayer's hematoxylin. Subsequently, standard procedure was used to dehydrate the slides and seal with coverslips. Negative controls were performed by omitting STS antibody during the primary antibody incubation. Normal placental tissue served as positive control.

Interpretation of steroid sulfatase immunostaining

Immunohistochemical staining of STS was evaluated semi-quantitatively based on staining intensity and percentage of positive cells. The intensity score was based on the staining intensity as negative (0 point), weak (1 points), intermediate (2 points), and strong (3 points). A final score was then calculated by a labeling index with percentage of positive cells.¹⁰ Expression of STS was considered ‘positive’ when >5% of tumor cells had any cytoplasmic staining.^{10,11}

Statistical analysis

Group wise comparisons of categorical clinical characteristics were by the chi-square and Fisher’s exact tests. For continuous variables, mean values were compared between the groups using the Mann-Whitney U-test. Disease-free survival (DFS) and overall survival (OS) were estimated using the Kaplan-

Meier method, and hazard ratio estimates and confidence intervals (CI) were generated using the Cox proportional hazard model. Statistical significance was defined as 2-sided *p* value of <0.05. Data were analyzed using SPSS software for Windows (version 18.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Fifty-nine patients were included in this study. Of these, 16 patients (27.1%) were STS positive (group A) (Fig. 1A) and 43 patients were STS negative (group B) (Fig. 1B). There was no significant difference in general characteristics between group A and group B. None of the patients received CCRT as an adjuvant treatment (Table 1). In the STS positive group, 8 patients were diagnosed as stage IA, and 3 patients were diagnosed as

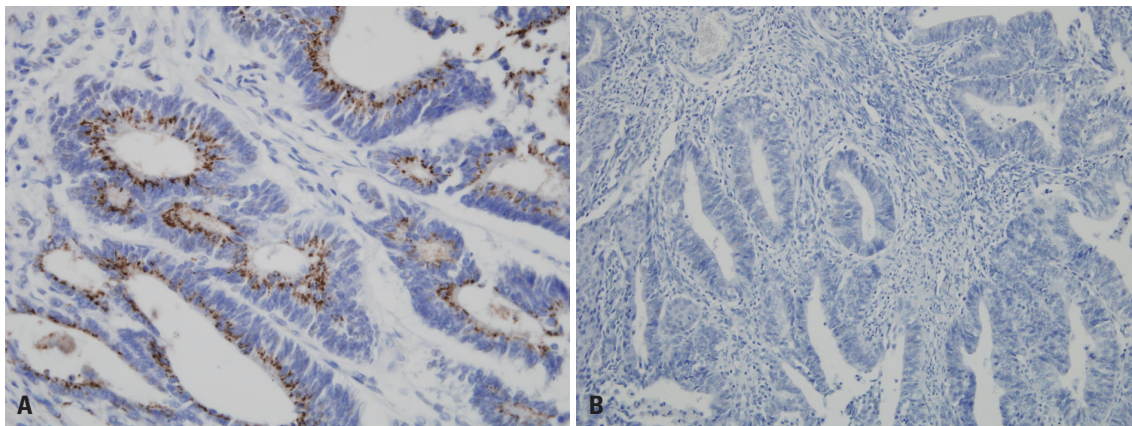


Fig. 1. The results of immunohistochemical staining (×400) of steroid sulfatase. (A) Positive. (B) Negative.

Table 1. General Characteristics in Patients with Endometrial Cancer

	STS positive (n=16)	STS negative (n=43)	<i>p</i> value
Age (median), range	55.5 (29–78)	58.0 (33–81)	0.97
Parity (median), range	2 (0–6)	3 (0–7)	0.81
Types of surgery			0.89
Hysterectomy+BSO	6 (37.5%)	17 (39.5%)	
Hysterectomy+BSO+lymphadenectomy	10 (62.5%)	26 (60.5%)	
FIGO stage			0.66
Stage I	11 (68.6%)	32 (74.4%)	
Stage II–IV	5 (31.2%)	11 (25.6%)	
Cell type			0.12
Endometrioid adenocarcinoma	16 (100%)	37 (86.0%)	
Serous adenocarcinoma	0 (0%)	6 (14.0%)	
Tumor grade			0.58
Grade 1	3 (18.8%)	11 (25.6%)	
Grade 2–3	13 (81.2%)	32 (74.4%)	
Adjuvant treatment			
None	8 (50.0%)	26 (60.5%)	
Chemotherapy	4 (25.0%)	5 (11.6%)	
Radiation therapy	4 (25.0%)	12 (27.9%)	

STS, steroid sulfatase; BSO, bilateral salpingo-oophorectomy; FIGO, International Federation of Gynecology and Obstetrics.

more than stage IB who were received adjuvant treatment; three patients were grade 1, 5 patients were grade 2 and 8 patients were grade 3. In the STS negative group, 26 patients were stage IA, and 6 patients were stage IB and IC who were re-

ceived adjuvant treatment; eleven patients were grade 1 and 15 patients were grade 2 and 6 patients were grade 3.

Two of the 16 patients in group A had recurrence and 1 patient died. Six patients had recurrence and subsequent mor-

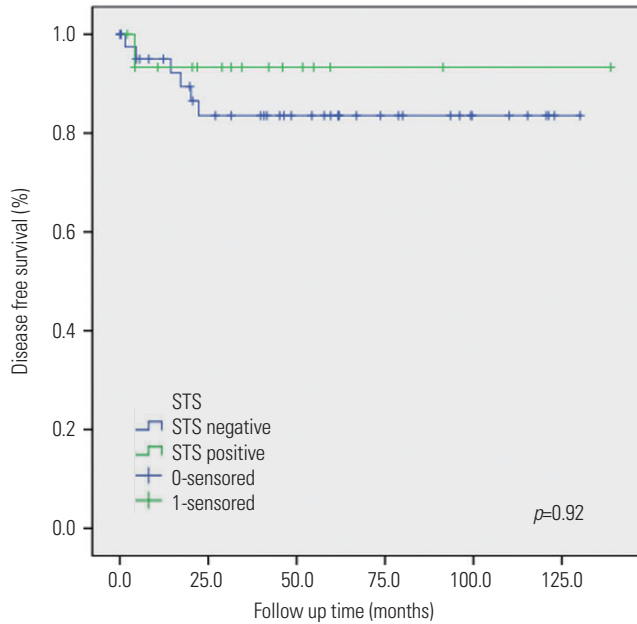


Fig. 2. Kaplan-Meier estimates of disease free survival between STS positive and STS negative in patients with endometrial cancer. STS, steroid sulfatase.

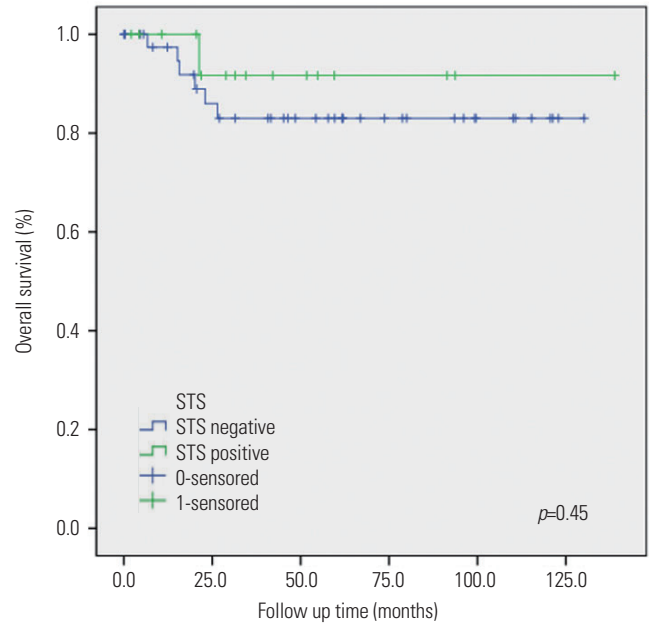


Fig. 3. Kaplan-Meier estimates of overall survival between STS positive and STS negative in patients with endometrial cancer. STS, steroid sulfatase.

Table 2. Association of Clinicopathologic Variables with Disease-Free Survival in Patients with Endometrial Cancer

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
Age			0.58			0.72
<50	1			1		
≥50	1.0	0.92–1.05		1.4	0.22–9.0	
Types of surgery			0.23			0.76
Hysterectomy+BSO	1			1		
Hysterectomy+BSO+lymphadenectomy	0.6	0.27–1.36		1.3	0.21–9.03	
FIGO stage			0.01			0.16
Stage I	1			1		
Stage II–IV	9.6	1.8–49.9		4.7	0.6–41.4	
Cell type			0.81			0.15
Endometrioid	1			1		
Serous	0.9	0.31–2.49		0.1	0.02–1.89	
Tumor grade			0.54			0.96
Grade 1	1			1		
Grade 2–3	1.9	0.2–16.2		1.1	1.2–9.4	
Adjuvant treatment			0.03			0.35
None	1			1		
Chemotherapy or radiation therapy	9.9	1.2–82.9		3.8	0.2–62.9	
STS			0.47			0.24
Negative	1			1		
Positive	0.5	0.06–3.8		0.3	0.3–2.4	

STS, steroid sulfatase; HR, hazard ratio; CI, confidential interval; BSO, bilateral salpingo-oophorectomy; FIGO, International Federation of Gynecology and Obstetrics.

tality in group B. Using Kaplan-Meier survival analysis, DFS (mean±standard deviation) was 129.83±8.67 (95% CI: 112.84–146.82) months in group A, and 111.06±7.17 (95% CI: 97.01–125.10) months in group B ($p=0.92$) (Fig. 2). OS (mean±standard deviation) was 129.01±9.38 (95% CI: 110.63–147.38) months in group A, and 111.16±7.10 (95% CI: 97.24–125.07) months in group B ($p=0.45$) (Fig. 3).

In univariate Cox proportional hazard analysis, FIGO stage ($p=0.007$ in DFS, $p=0.006$ in OS) and adjuvant treatment ($p=0.034$ in DFS, $p=0.034$ in OS) were significantly associated with DFS and OS. In multivariate analysis, however, FIGO stage and adjuvant treatment were not significantly associated with DFS and OS. The expression of STS was not statistically significant with DFS and OS (Table 2 and 3).

DISCUSSION

STS is a single enzyme that hydrolyses not only estrone sulfate (E1S) and estradiol sulfate (E2S), but also various steroid sulfates, such as dehydroepiandrosterone sulfate (DHEA-S) and cholesterol sulfate. Synthesis of sex hormones involves the conversion of androstenedione or testosterone to E2 or estrone (E1) by aromatase, and these hormones are converted by 17 β -hydroxysteroid dehydrogenase (17 β HSD). The inhibition of aromatase or 17 β HSD at this point would inhibit the synthesis of E2 or E1. However, Adiol, which is synthesized from DHEA

or DHEA-S via STS, is not inhibited and is eventually converted into estrogen.

The activity of STS is 12 times higher in endometrial cancer tissue, as compared to normal endometrial tissue,¹² and is expressed up to 86% on immunohistochemical staining.¹³ Previous studies showed that the STS pathway is an important source of estrogen and STS inhibitors are effective in breast cancer. These results have led to the clinical use of STS and STS inhibitors in patients with another hormone-dependent cancer (i.e., endometrial cancer).

STX 64 is the only STS inhibitor that completed the phase I clinical trial among the developed STS inhibitors. In various *in vivo* tumor models, STX 64 showed a great inhibitory effect of estrogen activity.¹⁴ STX 213 is a second generation STS inhibitor that showed an eight times stronger effect than STX 64 *in vitro* to completely block estrogen activity. The most significant characteristic of second generation STS inhibitor is the long duration of STS inhibition. In the study with a mouse model, the time to recover of STS activity was 10 days in STX 213, as compared to 3 days in STX 64.^{15,16} However, the clinical application of STS inhibitors is limited. DFS was reported from 2.8 months to 7 months in phase I of clinical trial with 14 breast cancer patients using STX 64.¹⁷

Although limited, clinical studies were conducted in breast cancer patients, but only animal studies were conducted in endometrial cancer patients. When STX 64 and STX 213 were administered orally to ovariectomized rats with endometrial

Table 3. Association of Clinicopathologic Variables with Overall Survival in Patients with Endometrial Cancer

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
Age			0.62			0.26
<50	1			1		
≥50	0.6	0.14–2.76		0.5	0.03–2.61	
Types of surgery			0.79			0.21
Hysterectomy+BSO	1			1		
Hysterectomy+BSO+lymphadenectomy	0.8	0.18–3.63		0.2	0.02–2.46	
FIGO stage			0.01			0.13
Stage I	1			1		
Stage II–IV	9.9	1.9–51.2		10.35	0.49–2.18	
Cell type			0.81			0.18
Endometrioid	1			1		
Serous	0.9	0.31–2.49		0.18	0.01–2.26	
Tumor grade			0.53			0.99
Grade 1	1			1		
Grade 2–3	1.9	0.24–16.3		1.0	0.1–9.2	
Adjuvant treatment			0.03			0.35
None	1			1		
Chemotherapy or radiation therapy	9.9	1.2–82.8		3.8	0.2–62.6	
STS			0.46			0.18
Negative	1			1		
Positive	0.5	0.05–3.7		0.2	0.03–1.9	

STS, steroid sulfatase; HR, hazard ratio; CI, confidential interval; BSO, bilateral salpingo-oophorectomy; FIGO, International Federation of Gynecology and Obstetrics.

cancer xenograft, the cancer cell growth was inhibited by 48% and 67%, respectively. Furthermore, cancer cell growth was inhibited up to 59% and serum estradiol was significantly reduced by STX 213 at 10 mg/kg daily.¹⁸

Unlike previous *in vitro* and *in vivo* studies, we used human endometrial cancer tissues. In this study, STS was expressed in sixteen patients out of 59 patients (27%), which was lower than the previous animal study with immuno-histochemical staining (86%). A possible explanation for this discrepancy might be the use of paraffin-embedded tissue post-formalin fixing vs. fresh endometrial cancer tissue. The use of rabbit polyclonal anti-STX antibody for STS detection might be another cause. Although previous study showed that the immune response was lower in the endometrium, as compared to human breast or hepatic tissue,¹⁹ there is no antibody with better response to STS detection.

Our results showed that the expression of STS did not affect DFS and OS in endometrial cancer patients. Although the effect was not statistically significant, survival was better in the STS positive group, unlike in the previous study conducted in breast cancer patients. In addition, FIGO stage and adjuvant treatment, which are known prognostic factors, were not associated with DFS and OS. The main reasons for this were the small sample size and the few events of recurrence and death. Other reasons were subtle differences between the two groups in the proportions of the cell types and in the numbers of patients receiving radiation therapy. Specifically, the STS negative group contained a larger number of patients with papillary serous adenocarcinoma and undergoing radiation therapy, and a previous study has shown that papillary serous adenocarcinoma has a poorer prognosis than endometrioid adenocarcinoma.²⁰ Also, radiation therapy has been considered the standard of adjuvant treatment, but the treatment failure rate in advanced stage patients is reportedly up to 67%.²¹

Another consideration is the sex hormone inhibitor with different response in breast and endometrium tissue. Tamoxifen has an anti-estrogen effect on breast tissue, but has an estrogen effect on endometrium tissue, which can cause endometrial hyperplasia or dysfunctional uterine bleeding and endometrial cancer.²²⁻²⁴ On the other hand, raloxifene, another selective ER active substance, has a treatment effect on breast cancer and neutral effect on endometrial tissue.²⁵ AIs also showed excellent clinical results in breast cancer, but there is only a minimal inhibitory effect on endometrial cancer.²⁶⁻²⁸ These results can be explained by ER. The subtype of ER is divided into alpha receptor (ER α) and beta receptor (ER β), both of which have a different distribution in each organ.²⁹ ER α is dominant in breast tissue, while ER β is dominant in endometrial tissue.³⁰ Therefore, the effect of the same sex hormone inhibitor can differ in breast and endometrial cancer. Another study showed that the mutation of exon 5, 8, and 36 in ER α may lead to lower anti-cancer effects in endometrial cancer.³¹⁻³³

Theoretically, the clinical effect of STS inhibitor in endome-

trial cancer is expected to be significant. The effect would be greater if there is concurrent inhibition of aromatase and 17 β HSD in the main pathway of estrogen synthesis and STS. However, we did not show the effect of the expression of STS in endometrial cancer as a prognostic factor. Although these findings were rather disappointing, they warrant future endometrial cancer trials with STS including ER, more specific antibody and endometrial cell biological markers.

In conclusion, STS was detected in approximately 27% human endometrial cancer tissue by immuno-histochemical staining. However, STS expression was not significantly associated with DFS and OS. Therefore, STS as a prognostic factor in patients with endometrial cancer is questionable, hence requires more study and a careful result-based approach.

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