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Female Sex Hormone Receptor Profiling in Uterine Adenosarcomas

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Introduction

First reported in 1974, uterine adenosarcomas (AS) were described as rare uterine tumors with both benign epithelial and low-grade sarcomatous components¹. Patients with uterine AS commonly present with abnormal uterine bleeding. These tumors are usually found within the uterine corpus or cervix, but have also been reported in extra-genital locations^{2–4}. When diagnosed at an early stage, these tumors can be managed surgically and are generally associated with a favorable prognosis. Conversely, adenosarcomas with sarcomatous overgrowth (AS+SO) contain >25% pure sarcoma, behave in a more aggressive fashion, and are associated with worse clinical outcomes^{5–8}. AS+SO often display high-grade histopathologic features with marked cytologic atypia, with or without heterologous elements. The immunoprofiling of AS has revealed a greater prevalence of estrogen receptor alpha (ER α) and progesterone receptor (PgR) expression when compared to AS+SO⁹. In contrast, AS+SO display a loss of ER/PgR expression, an increased Ki-67 proliferation index, and loss of CD10 expression¹⁰.

Expression of different subtypes of estrogen receptor (ER) in various tumors has been described. For example, in ovarian carcinoma, loss of estrogen receptor beta (ER β) expression, a classical nuclear estrogen receptor homolog of ER α , has been linked to the development of the disease¹¹. Recently, expression of an ER β isoform localized to the cytoplasm had been described to be associated with a decreased overall survival and increasing rates of chemoresistance¹². An additional estrogen receptor of the 7-transmembrane G protein-coupled receptor family, the G protein-coupled estrogen receptor (GPER) reportedly interacts with estrogen independently of the classic receptors^{13, 14, 15, 16}.

GPER has been shown to have complex effects in different tumor types with both stimulatory and inhibitory actions. Interestingly, GPER is stimulated by several different ligands in addition to estrogen, including the anti-estrogens such as tamoxifen and raloxifene^{13, 17}. In breast cancer, both its overexpression and downregulation have been linked to advanced disease and poor prognosis¹⁸. In endometrial cancer, GPER expression has been inversely associated with the expression of ER α ¹⁹. Clinically, the increased expression of GPER has been linked to aggressive uterine tumors, and shown to be associated with advanced stage disease²⁰.

The variable prognosis in patients with uterine AS is dependent upon stage at diagnosis and the presence of sarcomatous overgrowth and/or heterologous elements. There is limited data regarding treatment of these tumors in the adjuvant and recurrent settings, as they are rare. Identification of ER β and/or GPER positivity, which has been associated with advanced disease and poorer outcomes in other gynecologic cancers, may potentially add prognostic information for patients with uterine AS. The aim of this study was to analyze the differential estrogen receptor status, including ER α , ER β , and GPER, as well as PgR status in uterine AS.

Materials and Methods

From the period of August 2001 to November 2013, eleven patients with histologic diagnoses of uterine AS were identified in our multi-campus, single institution (Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, New York) database. The Montefiore Office of Research/Institutional Review Board approved the study and clinical data was abstracted from the medical records. All tumors histologically identified as uterine AS, including those with sarcomatous overgrowth and heterologous elements, were included. Patients at all stages of disease were included in this study. In one case, tissue samples were obtained from both the initial tumor, as well as a recurrent tumor several years later. Not all patients underwent hysterectomy at our institution. The only cases excluded were those without sufficient tissue available for further immunohistochemical evaluation.

Paraffin embedded and formalin fixed tissue blocks were sectioned at 4 μ m thickness and stained by hematoxylin and eosin (H&E). They were reviewed by a gynecologic pathologist (RGK) to confirm adequate quantity of tumor sample for immunohistochemical work-up. Tumors were classified as AS if the sarcomatous component displayed a monotonous tumor cell population with low-grade nuclei, low mitotic activity, and absence of necrosis. The AS +SO was characterized by pure sarcoma comprising more than 25% of the tumor tissue. Tumors that did not fit the criteria for AS+SO were classified as AS with high-grade features, if marked nuclear pleomorphism, increased mitotic activity including the presence of atypical mitoses, necrosis, and/or heterologous elements were present. Heterologous elements were defined as those comprised of osteosarcoma, chondrosarcoma or rhabdomyosarcoma components. Once the histologic diagnosis was confirmed, representative tumor tissue sections were stained for ER α (Clone 1D5; Dako North America, Carpinteria, CA), ER β (Clone 14C8; GeneTex, San Antonio, TX), and PgR (Clone PgR 636; Dako North America) utilizing standard immunohistochemistry protocols as previously described²¹. Staining for GPER, utilizing a rabbit polyclonal affinity-purified

antibody directed against the C-terminus, was performed by our collaborators at the University of New Mexico Health Sciences as previously described²².

The immunostained tumor sections were then examined for the percentage of overall cells stained and intensity of antibody staining by the study pathologist (RGK). The staining intensity was scored as weak, moderate, or strong. The product of these two values was then used to calculate the H-score.

Descriptive statistics were calculated using clinicopathologic data abstracted from the medical records. Unpaired t-test was used to compare the mean H-scores of receptor expression between AS and AS+SO/AS with high-grade features. Analyses were performed with STATA version 13.1 (College Station, TX) as well as GraphPad Prism version 6 (GraphPad Software Inc, San Diego, CA).

Results

Over a twelve-year period, eleven patients were diagnosed with uterine AS at our institution, and clinical information was available for ten of these patients. The demographic and clinical characteristics are described in Table 1. There were seven cases of AS and three cases of adenosarcoma with high-grade features. Of the cases with high-grade features, two displayed sarcomatous overgrowth and of those, one contained heterologous elements. One case bordered on sarcomatous overgrowth with high-grade cytomorphology. The mean body mass index (BMI) was 34.5 kg/m², meeting the Institute of Medicine Class I obesity definition. All patients were parous and had at least one delivery with a range of 1–12 deliveries. Most patients were menopausal (80%) and presented with postmenopausal bleeding (70%). Specimens obtained were from surgical resection, nine patients underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and only one underwent limited surgery (dilation and curettage). Most patients presented with Stage I disease. Breakdown of stage based on the FIGO 2009 staging system was as follows: 9 (90%) Stage I and 1 (10%) Stage III. One patient underwent chemotherapy and three received radiation as adjuvant treatment. Three patients recurred, including one who recurred twice and the specimen from the first recurrence was evaluable for staining and analysis.

The hormone receptor profile of each tumor sample is described in Table 2. In AS, seven of eight tumor samples showed moderate or strong intensity immunostaining for ER α . In contrast, all tumors with high-grade features (both AS+SO and the sample with high-grade cytomorphology) showed minimal to no ER α immunoreactivity. Immunostained tumor sections are shown in Figure 1. As summarized in Figure 2, there was a significant decrease with respect to ER α H-scores in those with high-grade features ($p=0.01$). Relative to ER α , the PgR immunostaining intensity showed greater variability among tumor samples; however, significantly lower PgR H-scores were observed in tumors with high-grade features ($p=0.04$, Figure 2). In one patient with AS+SO and recurrent disease after 2 years, the recurrent tumor remained negative for ER α and PgR. Results of immunostains for estrogen receptors ER β and GPER were similar across all samples. ER β immunostaining was variable with respect to percentage of cells staining, but was detectable in all tumor samples except one case. In that outlier case (patient #5), immunostaining was undetectable

for all of the hormone receptors. Immunostaining for GPER was absent in the majority of tumor samples, although three samples (two AS, one AS+SO with heterologous elements) showed moderate to strong staining.

Discussion

To our knowledge, this is the first study to comprehensively evaluate the female sex hormone receptor profile in uterine adenosarcomas. In this study, adenosarcoma with high-grade features (with or without sarcomatous overgrowth) demonstrated loss of ER α and PgR expression. The decreased expression of ER α and PgR in this group was statistically significant, despite the small sample size, and is consistent with a previous study. A multi-institutional study examining ER/PgR expression has found 85% positivity for ER and 80% positivity for PgR in AS without sarcomatous overgrowth. Those with sarcomatous overgrowth were ER positive in 50% and PgR positive in 25% of cases. There was also less staining reported in the sarcomatous components of both tumors with and without SO⁹. A subsequent analysis evaluated ER/PgR immunostaining in AS with and without SO. The AS+SO tumors displayed a median ER labeling index of <5%, while diffuse ER expression of >80% was seen in those without sarcomatous overgrowth. Similarly, PgR positivity was less frequently observed in tumors with AS+SO than those without (20% vs. >80%)¹⁰. We noted that the hormone receptor profile of the case with high-grade cytomorphology closely resembled adenosarcomas with sarcomatous overgrowth, suggesting the biology of tumors with high-grade cytomorphology may be more similar to those with sarcomatous overgrowth than those without. According to one of the previous studies of uterine carcinosarcoma, increased expression of ER β and GPER in the glandular component of these higher-grade tumors was associated with advanced stage disease¹⁶. However, our study showed similar levels of ER β expression in uterine AS. No significant differences in GPER expression were detected between AS versus AS+SO. GPER expression was detected in AS+SO tumor that also contained heterologous elements, but not in the remaining two samples with high-grade features. As there was only one patient with a tumor containing heterologous elements and positive staining for GPER, limited conclusions can be drawn. We found that the GPER and ER β expression were not significantly linked to histological subtype, suggesting that these receptors are not drivers of the more aggressive behavior of adenosarcomas with high-grade features or sarcomatous overgrowth.

Limited to smaller studies and case series, the literature has shown an overall favorable prognosis in patients with early stage uterine adenosarcoma, with 5-year overall survival of approximating 83–84%^{6–8}. Those with deeply myoinvasive disease, stage IB or greater had a lower rate of survival, ranging from 63–69%. The majority of patients with metastatic disease outside of the uterus fare poorly, with 0–50% survival rates^{6, 8}.

In our study, we had a limited number of patients with AS+SO. A multi-institutional study looking at treatment outcomes found patients whose tumors exhibited sarcomatous overgrowth were more likely to recur, although this did not reach statistical significance. Of those with AS+SO who received adjuvant treatment, patients undergoing radiation therapy did not experience any recurrences within the treatment field and of those who experienced distant recurrences, they were most common in the lung⁸. Additional investigators have

found few responses to sarcoma-based treatment and even more limited responses to hormone based therapies, although ER/PgR tumor status was not consistently known for all patients⁷.

In this study, the comprehensive estrogen receptor profiling is novel and contributes new insights into this rare tumor subtype. The major limitation to this study is the small sample size, with even fewer tumors exhibiting high-grade features, limiting our hypothesized detection of differential hormone receptor expression amongst AS and AS+SO. Future larger studies with the inclusion of a cohort of cases, particularly those containing sarcomatous overgrowth and/or heterologous elements, could potentially increase the detection of hormone receptor differences among AS subtypes and help to gain further insights into the biology of this tumor.

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Figure 1. Expression of ER α , ER β and GPER among AS and AS+SO

A. AS (H&E, 10X) B. AS with high-grade features/cytomorphology (H&E, 10X) C. AS, monotonous low-grade sarcoma histomorphology (H&E, 20X) D. AS, nuclear expression of ER α (20X) E. AS+SO, pleomorphic high-grade sarcoma histomorphology (H&E, 20X) F. AS+SO, nuclear expression of ER β (20X) G. AS+SO, high-grade nuclei with frequent mitoses (H&E, 40X) H. AS+SO, predominantly cytoplasmic (and nuclear) expression of GPER (20X).

Figure 2. H-Scores for Receptor Staining

H-scores (mean±SD) of each receptor type (ER α , PgR, ER β and GPER) for AS and AS+SO tumors with high-grade features (high-grade cytomorphology and sarcomatous overgrowth). Significant differences in H-scores were only observed with ER α and PgR.

Table 1

Characteristics of patients with uterine adenosarcomas (AS)

Variable	N=10(%)
Age at diagnosis (years)	59.4 ± 11.9
BMI (kg/m ²)	34.2 ± 8.5
Parity, mean	2
Menopausal	8 (80)
Prior history of radiation therapy for treatment of other malignancy	1 (10)
Postmenopausal bleeding	7 (70)
FIGO 2009 Stage	
IA	7 (70)
IB	2 (20)
IIIA	1 (10)
Heterologous elements present	1 (10)
Sarcomatous overgrowth present	2 (20)

Table 2

Hormone receptor profile of all tumor samples of AS

Case	Histology	Stage	ER alpha		ER beta		GPER		Hscore		Intensity		PgR	
			Intensity	%	Intensity	%	Intensity	%	Hscore		Intensity		%	Hscore
1	AS	IA	3	98	3	98	0	0	294		0	3	98	294
2	AS	IA	3	90	2	90	0	0	180		0	3	90	270
3	AS	IA	3	95	1	5	0	0	5		0	3	80	240
4	AS	IA	3	90	1	35	0	0	35		0	3	80	240
5	AS	IB	0	0	0	0	0	0	0		0	0	0	0
6	AS	IA	3	75	1	10	2	30	10		2	3	95	285
7	AS	IA	2	50	1	40	0	0	40		0	3	60	180
8	AS	Unstaged	3	60	1	10	3	80	10		3	0	0	0
9	AS with high-grade cytomorphology	IB	0	0	1	15	0	0	15		0	2	5	10
10	AS+SO	IA	1	5	2	40	2	15	80		2	3	15	45
11	AS+SO	IIIA	0	0	2	95	0	0	190		0	0	0	0
	AS+SO	Recurrence	0	0	1	30	0	0	30		0	0	0	0