Unique Molecular Signatures Between High-Grade and Low-Grade Endometrial Stromal Sarcoma: An Analysis of 96 Cases

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Abstract

Objective: Endometrial stromal sarcoma (ESS) is a rare form of uterine cancer, traditionally categorized as high-grade (HG) or low-grade (LG) ESS. Molecular and genomic changes that underlie the distinct clinical characteristics associated with each subtype are largely uncharacterized. We aim to identify genomic and protein expression differences between high-grade and low-grade tumors in a large cohort of ESS.

Methods: Out of 3133 uterine cancers submitted for a molecular profiling test from March 2011 to July 2014, 143 ESSs were identified based on reported pathology. Testing was ordered per physician request and included a combination of sequencing (Sanger or next generation sequencing), protein expression (immunohistochemistry), and /or gene amplification (FISH/CISH).

Results: Of 144 ESSs, 52 (36%) were HGESS, 44 (31%) LGESS, and 47 (33%) unspecified. Compared with HGESS, patients with LGESS were on average 2 years younger (54.7 vs 56.9). One out of 47 genes sequenced, only one mutation, a variant of unknown significance in JAK3, was detected among LGESS, compared to 16 mutations in HGESS. Among HGESS, TP53 was the most common mutation at 32%, compared to 0% within LGESS (p=0.02). Hormone receptor expression was significantly greater in LGESS than HGESS: ERα (90% vs 21%), PR (86% vs 21%) and AR (60% vs 17%), respectively (p<0.001). 69% of HGESS were ER and PR negative while only 7% of LGESS were ER and PR negative. EGFR expression was common in both low and high grade ESS (88% and 73%). Loss of PTEN was more common in HGESS (40% vs 17%, p=0.01), suggesting potential utility of inhibitors of the PI3K pathway. Increased TOP2a expression, associated with higher proliferation and anthracycline efficacy, was more common in HGESS (87% vs 26%, p<0.001). A significant higher proportion of HGESS patients expressed TS and RRMI, known to confer resistance to folate analogue and gemcitabine, respectively (75% vs 28% TS, p<0.001 and 52% vs 26% RRM1, p=0.025, respectively).

Conclusions: Our findings suggest HGESS and LGESS have distinct molecular signatures. LGESS rarely carry mutations and are hormonally active, suggesting potential utility with fertility preservation and endocrine therapy. HGESS are largely hormonally independent with frequent TP53 mutation. Anthracyclines, and drugs targeting the PI3KCA pathway may warrant consideration in a subset of patients with HGESS.

Background

- Uterine sarcomas are a rare form of endometrial cancer arising from the connective tissue (stroma) of the endometrium
- Uterine sarcomas account for approximately 3% of all uterine malignancies [1, 2]
- Endometrial stromal sarcomas (ESS) are one the most common types of uterine sarcomas and are traditionally categorized as either high-grade (HG) or low-grade (LG) ESS [3]
- Compared with other uterine malignancies, ESS affects younger women with mean age from 42-58 years [3].
- LG ESS are typically indolent tumors with late recurrences, while HG ESS behave as high grade sarcomas and carry a poor prognosis [4].
- Molecular and genomic changes that underlie the distinct clinical characteristics associated with each subtype are largely uncharacterized
- Broader understanding of the molecular and genomic characteristics could provide alternative treatment options with targeted therapies

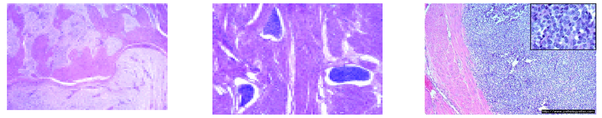


Figure 1. H&E Stains [5].
(a) LG ESS
(b) LG ESS
(c) HG ESS

Methods

- 3133 cases of endometrial cancers were submitted to Caris Life Sciences from March 2011 to July 2014.
- Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC) and gene amplification (CISH or FISH).
- IHC analysis was performed on formalin-fixed paraffin-embedded tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, and AutostainerLink 48, Dako), and commercially available antibodies.
- Fluorescent in-situ hybridization (FISH) was used for evaluation of the HER-2/neu [HER-2/CEP17 probe], EGFR [EGFR/CEP7 probe], and cMET [cMET/CEP7 probe] (Abbott Molecular/Vysis). HER-2/neu and cMET status were also evaluated by chromogenic in-situ hybridization (INFORM HER-2 Dual ISH DNA Probe Cocktail; commercially available cMET and chromosome 7 DIG probe; Ventana). The same scoring system was applied as for FISH.
- Direct sequence analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina MiSeq platform. Specific regions of 47 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel.
- Mutation analysis by Sanger sequencing included selected regions of BRAF, KRAS, NRAS, c-KIT, EGFR, and PIK3CA genes and was performed by using M13-linked PCR primers designed to amplify targeted sequences.
- Retrospective data analysis; Statistical analysis (unpaired t-tests used to compare biomarker expression across histologic subtypes) performed using Prism™ v6. Biomarker associations were calculated by two-tailed Fisher Exact tests.

Results

Table 1. Age distribution of Endometrial Stromal Sarcoma

Cancer type	N (%)	Average Age	Range
Endometrial stromal sarcoma	143	56.2	22-83
High grade	52 (36.3)	56.9	22-83
Low grade	44 (30.8)	54.7	37-81
unspecified	47 (32.9)	56.7	35-79

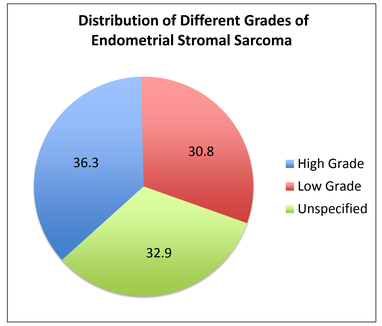


Figure 2: Comparison of molecular differences between HG and LG-ESS

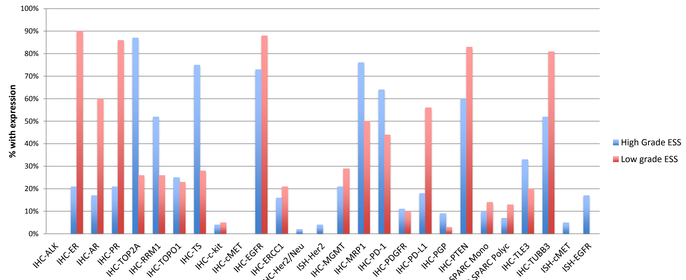
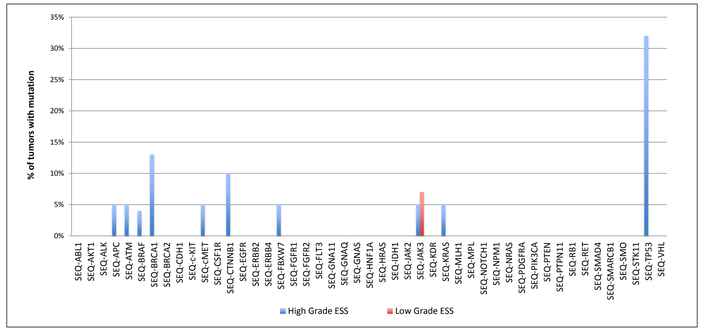


Table 1: Summary of significant molecular distinctions between HG and LE-ESS

Marker (IHC)	Pos	Neg	Total	High Grade ESS	Pos	Neg	Total	Low grade ESS	P value
IHC-ER	11	41	52	21%	38	4	42	90%	<0.0001
IHC-Androgen Receptor	9	43	52	17%	26	17	43	60%	<0.0001
IHC-PR	11	41	52	21%	36	6	42	86%	<0.0001
IHC-TOP2A	40	6	46	87%	10	28	38	26%	<0.0001
IHC-RRM1	24	22	46	52%	10	28	38	26%	0.025
IHC-TS	36	12	48	75%	11	28	39	28%	<0.0001
IHC-PTEN	31	21	52	60%	35	7	42	83%	0.0138
IHC-TUBB3	11	10	21	52%	13	3	16	81%	0.0912

Results (continued)

Figure 3: Comparison of mutation differences between HG (N=20) and LG-ESS (N=14)



Conclusions

- We identified several pathways that warrant further exploration in the histologic subtypes of a relatively large cohort (n=96) of endometrial stromal sarcomas
- There is a significantly higher frequency of hormone receptor expression in low grade ESS suggesting potential benefit with hormone therapy
- The proliferation marker TOPO2A was significantly higher in high-grade ESS
- TS and RRM1 were more often expressed in high-grade ESS
- TP53 was exclusively and very frequently mutated in high-grade ESS
- Low-grade ESS carried no mutations except for on JAK3 mutation which is a variant of unknown significance
- Overall we identified differential molecular profiles within high-grade and low-grade ESS that could guide future therapy.
- Correlating molecular profiles with clinical outcomes will assist in developing rational guidelines for therapy in individuals with ECC

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