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Molecular markers in recurrent stage I, grade 1 endometrioid endometrial cancers

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HIGHLIGHTS

- Recurrences in stage I, grade 1 endometrial cancer are infrequent but confer a poor prognosis.
- Recurrent cases have significantly higher rates of *CTNNB1* mutations and MSI-H than non-recurrent controls.
- Among recurrent cases, *CTNNB1* mutations most frequently occur in MSS tumors.
- *CTNNB1* and MSI-H should be further evaluated as possible molecular markers in risk-stratification of endometrial cancer.

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ABSTRACT

Objectives. Stage I, grade 1 endometrial cancers have low recurrence rates and often do not receive adjuvant therapy. We compared recurrent cases to matched non-recurrent controls to evaluate for molecular markers associated with higher risk of recurrence.

Methods. A case-control study including all cases of recurrent stage I, grade 1 endometrioid endometrial cancer at one institution in a ten-year period. Cases were matched to controls by age, BMI, weight and stage. Molecular testing and immunohistochemistry were performed on archival tumor specimens: microsatellite instability (MSI-H), mismatch repair status, *POLE* mutational status, and next-generation sequencing.

Results. 15 stage I, grade 1 endometrial cancer cases with recurrent disease and available tumor specimens were identified. *CTNNB1* and MSI-H were present at significantly higher rates in cases than controls (*CTNNB1* 60% vs. 28%, OR 3.9, 95%CI 1.1–14.7, $p = 0.04$ and MSI-H 53% vs. 21%, OR 4.4, 95%CI 1.1–17.0, $p = 0.03$). POLE mutations were found in 0% of cases vs. 7% of controls ($p = 0.54$). Among specimens demonstrating microsatellite stability (MSS), 100% of cases vs. 26% of controls had *CTNNB1* mutations ($p < 0.001$). *CTNNB1* wild type tumors were MSI-H in 100% of cases vs. 19% of controls ($p < 0.001$).

Conclusions. Compared to controls, *CTNNB1* mutation is present at significantly higher rates in recurrent stage I, grade 1 endometrial cancers and is found most commonly in MSS tumors. MSI-H is also present at significantly higher rates in recurrent cases. These markers may be useful for prognostic risk stratification and adjuvant therapy decision-making in this otherwise low-risk population.

1. Introduction

Endometrial cancer incidence and mortality are on the rise worldwide [1–3]. In the United States, endometrial cancer is the most common gynecologic cancer with an estimated 63,230 new cases in 2018 — an increase from 40,100 in 2008 [3,4]. Stage I, grade 1 endometrial cancer is generally perceived as a surgically managed disease with an excellent prognosis. Although 5-year survival for this group of tumors is over 90% [5], when they do recur, they have an especially poor prognosis and few treatment options [1]. Accurate risk-stratification is therefore critical to the survival of stage I, grade 1 endometrial cancer patients [1,6–8].

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The current clinical risk-stratification for endometrial cancer is based on patient age and histopathologic factors of the tumor including histology type, grade, surgical stage, tumor size, myometrial invasion and lymphovascular space invasion (LVSI). Multiple large studies including the Gynecologic Oncology Group (GOG) 33 trial, GOG 99 trial and Post Operative Radiation Therapy in Endometrial Cancer (PORTEC) trials have determined that these histopathologic factors are associated with a higher risk of recurrence and metastases in endometrial cancer. However, there is increasing evidence that risk-stratification of endometrial cancer using these histopathologic factors is not entirely accurate in predicting outcomes [8–13].

Recently, there is substantial evidence that molecular testing could significantly improve the risk-stratification and prognostication of endometrial cancer. Some of the most comprehensive data supporting molecular classification of endometrial cancer is from The Cancer Genome Atlas (TCGA) study [12], which identified four distinct genomic endometrial cancer subtypes with statistically different clinical outcomes. The molecular markers that defined these genomic subtypes included microsatellite instability (MSI-H), *CTNNB1*, *TP53*, and *POLE* hotspot mutations [1,6,12,14,15]. Multiple studies have evaluated the TCGA data and demonstrated reproducibility of the genomic subtypes and their associated prognoses, indicating that molecular classification of endometrial cancer has the potential to be a reliable and impactful addition to the clinical risk-stratification of endometrial cancer [14–19].

Among endometrial cancers, risk-stratification is especially difficult for early stage, low grade tumors. This population includes both tumors that are cured with surgery, requiring no adjuvant therapy, and tumors that have a higher risk of recurrence and therefore should be considered for adjuvant therapy. The current clinical risk-stratification models attempt to use histopathologic factors to determine which tumors among this early stage, low grade population have a higher risk of recurrence and should receive adjuvant therapy; however, as previously mentioned, these risk-stratification models may not provide a completely accurate prognostication [7–11,15]. For this reason, we used molecular testing to evaluate recurrences among early stage, low grade endometrial cancer. Our objective was to determine which molecular markers were associated with a higher risk of recurrence in stage I, grade 1 endometrioid endometrial cancers.

2. Methods

Medical records at a single institution from January 2007 through December 2017 were reviewed to identify cases of recurrent stage I, grade 1 endometrioid endometrial cancer. We included patients age 18 to 99 and excluded those with no available pathology specimen. This same medical record review was used to identify controls, which were defined as patients with stage I, grade 1 endometrioid endometrial cancer with no recurrence. All controls were required to have clinical surveillance with no evidence of recurrence for a duration at least six months longer than the longest time to recurrence of the cases. Controls were matched to cases in a 2:1 ratio and were matched by age, BMI, weight, stage.

Archival tumor pathology specimens were used for molecular testing. Molecular testing was performed at the Colorado Molecular Correlates Laboratory (CMOCO) and consisted of microsatellite instability testing via the Promega MSI Analysis kit (Promega, Madison WI), *POLE* mutational testing via a custom-designed Sanger sequencing assay, and mutational testing of 67 genes by a next-generation sequencing (NGS)-based assay For the NGS assay, library preparation was performed using the ArcherDx VariantPlex Solid Tumor kit (ArcherDx, Boulder, CO), sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA), and analysis of raw sequence data was performed using the ArcherDx Analysis platform (v5, ArcherDx). Mismatch repair (MMR) immunohistochemistry (IHC) was performed on archival tumor specimens to evaluate for deficiencies in *MLH1*, *PMS2*, *MSH2*, and *MSH6* markers.

All molecular variants noted as present by the bioinformatics analysis algorithm were carefully vetted by trained personnel before being reported. Several factors including variant allele frequency (VAF), read depth at the position, complexity in reads supporting the variant, read direction bias, strand bias, and sequencing noise in surrounding positions were considered in the process. The VAF cut-off used in our lab for visualizing and reporting a variant is 3%. Population polymorphisms were not reported.

Medical charts were abstracted for patient demographics, tumor pathologic characteristics, and operative details. Patient and tumor characteristics and the molecular testing results were compared using chi-square and Fisher's exact tests.

3. Results

We identified 311 women with stage I, grade 1 endometrioid endometrial cancer. Of those, 18 (6%) had recurrent disease. Of the 18 recurrent cases, 15 had available tumor specimens. None of these recurrent cases received adjuvant therapy following initial surgical management. Median time to recurrence among these 15 cases was 48 months and the longest time to recurrence was 80 months. Recurrence location was at the vaginal cuff in 8 of 15 cases (53%), pelvic lymph nodes in 3 of 15 (20%) and para-aortic lymph nodes or elsewhere in the abdomen in 4 of 15 (27%) (Table 1).

From the 311 women with stage I, grade 1 endometrioid endometrial cancer, we identified 30 matched non-recurrent controls. All 30 controls had clinical surveillance >86 months and no evidence of endometrial cancer recurrence. One of these controls had a poor tissue sample that was ultimately inadequate for molecular testing, resulting in 29 total controls with adequate tissue samples. Cases and controls were matched by age at diagnosis, BMI, weight and stage. Cases and controls also did not differ in surgical approach (Table 2).

Molecular testing results demonstrated that recurrent cases had a high frequency of *PTEN* (80%), *PIK3CA* (60%), and *CTNNB1* (60%) mutations, a low frequency of *TP53* mutations (7%), and no *POLE* hotspot mutations (0%). Controls had a high frequency of *PTEN* mutations (86%) and a low frequency of MSI-H (21%), *TP53* (14%), and *POLE* hotspot (7%) mutations. The frequency of both *CTNNB1* mutations and MSI-H was significantly higher among cases than controls (*CTNNB1* 60% vs. 28%, OR 3.9, 95%CI 1.1–14.7, $p = 0.04$ and MSI-H 53% vs. 21%, OR 4.4, 95%CI 1.1–17.0, $p = 0.03$). There was no significant difference in the frequency of any other molecular marker between cases and controls, including *PTEN*, *PIK3CA*, *KRAS*, *TP53* and *POLE* hotspot mutations (Table 3). The full mutational analysis is available in Table 5 in the supplemental material. Among specimens demonstrating microsatellite stability (MSS), 100% (7/7) of cases and 26% (6/23) of controls had *CTNNB1* mutations ($p < 0.001$). *CTNNB1* wild type tumors were MSI-H in 100% (6/6) of cases vs. 19% (4/21) of controls ($p < 0.001$). Among recurrent cases, 7 of 9 (78%) *CTNNB1* mutant tumors were MSS, and 6 of 8 (75%) MSI-H tumors were *CTNNB1* wildtype (Table 4).

4. Discussion

In this case-control study, we found that cases of recurrent stage I, grade 1 endometrioid endometrial cancer had a significantly higher rate of *CTNNB1* and MSI-H than matched non-recurrent controls. These findings indicate that these markers are associated with a risk of recurrence

Table 1
Recurrence location among 15 cases.

Recurrence location	Number, n	Percentage, %
Vaginal cuff	8	53%
Pelvic	3	20%
Abdominal	4	27%

Table 2
Characteristics of cases and controls.

Variable	Cases (n = 15)	Controls (n = 29)	*p* value
Age	57 (47–69)	59 (44–71)	0.53
BMI	33.1 (21.6–53.8)	34.2 (20.6–53.0)	1.00
Weight (kg)	83.5 (57.2–165.1)	87.1 (51.9–145.6)	1.00
Stage			0.60
IA	13 (87%)	27 (93%)	
IB	2 (13%)	2 (7%)	
Surgical route			0.21
Abdominal	8 (53%)	21 (72%)	
Minimally invasive	7 (47%)	8 (28%)	

in this low-risk tumor population and could therefore be considered for use in risk stratification of endometrial cancers.

CTNNB1 is a gene involved in the Wnt signaling pathway, which controls cell differentiation and proliferation. Mutations in the Wnt pathway and in *CTNNB1* specifically have been found to be associated with carcinogenesis in a number of different cancer types [7,20,21]. The finding in our study that *CTNNB1* mutation is associated with recurrence in endometrial cancer is supported by recent data looking at molecular classification of endometrial cancer. First, the TCGA study identified a distinct genomic subgroup of endometrial cancers described as copy-number low: endometrioid tumors characterized by high frequency of *CTNNB1* mutations, microsatellite stability, and low mutation rates [12]. A subsequent analysis of only endometrioid tumors involved in the TCGA study demonstrated that tumors with Wnt pathway activation and *CTNNB1* mutations specifically had a worse overall survival, especially in comparison to other low grade endometrioid tumors [20]. These findings were then reinforced by Kurnit et al., who demonstrated a significantly decreased recurrence-free survival in stages I–II and grades 1–2 endometrial cancers with *CTNNB1* mutations [7].

Deficiencies in the MMR genes *MLH1*, *PMS2*, *MSH2*, and *MSH6* can be the result of germline or somatic mutations. Tumors with MMR deficiencies have an accumulation of mismatched base-pairs in microsatellite regions and therefore a detectable change in the length of the microsatellite region, which is termed microsatellite instability, or MSI-H [22]. MMR deficiencies and MSI-H are a well-known event in endometrial cancer, present in up to 40% of endometrial cancer cases [6,23]. However, the impact of MMR deficiencies and MSI-H on outcomes is not well understood as previous studies have produced inconsistent findings on progression-free and overall survival. Our data, however, is congruent with the results of the GOG 210 trial, which is one of the largest studies evaluating the association of MMR deficiencies and endometrial cancer outcomes. The GOG 210 trial included endometrial cancers of all stages and grades and found that women with MMR deficiencies had lower progression-free survival than women with intact MMR (Hazard Ration 1.37, $p < 0.05$, 95%CI 1.00–1.86) [23].

Although there are a number of studies that have evaluated *CTNNB1* mutations, MMR deficiencies and MSI-H in endometrial cancers, our study is the first to do so in a low-risk population. With our study population being strictly defined as stage I, grade 1 endometrioid endometrial cancers that did not receive adjuvant therapy, we have eliminated incongruent variables that exist when evaluating endometrial cancers of a variety of stages and grades. Our study population is therefore one of our study's main strengths. The sample size of our population is small, due to the rarity of recurrence in stage I, grade 1 endometrioid endometrial cancers, but our case-control design allows for effective analysis of this small population.

Table 3
Frequencies of molecular markers in cases and controls in order of decreasing frequency.

	Cases (%)	Controls (%)	p value
PTEN	80	86	0.68
PIK3CA	67	48	0.34
CTTNB1	60	28	0.04
MSI-H	53	21	0.03
KRAS	27	35	0.74
PIK3R1	27	35	0.74
TP53	7	14	0.65
MLH1	7	3	1.00
POLE hotspot	0	7	0.54

Full list of mutational analysis available in Table 5 in supplemental material.

Table 4
Microsatellite stability and *CTNNB1* mutation status in cases and controls.

Cases, n = 15	MSS	*CTNNB1* wildtype
	7/15 (47%)	0/7 (0%)
		CTNNB1 mutant
		7/7 (100%)
		CTNNB1 wildtype
	MSI-H	6/8 (75%)
	8/15 (53%)	*CTNNB1* mutant
		2/8 (25%)
Controls, n = 29	MSS	*CTTNB1* wildtype
	23/29 (79%)	17/23 (74%)
		CTNNB1 mutant
		6/23 (26%)
		CTTNB1 wildtype
	MSI-H	4/6 (67%)
	6/29 (21%)	*CTNNB1* mutant
		2/6 (33%)

Our study is also unique in that we evaluate *CTNNB1* mutations and MSI-H in both their association with disease recurrence and their relationship with each other. Among recurrent cases, 100.0% of specimens demonstrating microsatellite stability (MSS) had *CTNNB1* mutations and 100.0% of specimens with *CTNNB1* wildtype demonstrated MSI-H. Also among recurrent cases, the large majority of *CTNNB1* mutant tumors had no MMR deficiencies or MSI-H (7 of 9, 78%), and the large majority of MSI-H tumors had no *CTNNB1* mutations (6 of 8, 75%). *CTNNB1* mutations and MSI-H are associated with a higher risk of recurrence in our population of stage I, grade 1 endometrioid endometrial cancers, but their associations with disease recurrence are clearly independent of each other, making the *CTNNB1* mutation and MSI-H independent risk factors.

The molecular data demonstrated in this study, in combination with TCGA data and other previously mentioned studies, supports the use of molecular markers in risk-stratification of endometrial cancer. Clinical risk-stratification is used to make critical adjuvant therapy decisions and therefore accurate prognostication is imperative. Current risk-stratification systems for endometrial cancers are based on age and histopathologic factors. However, there is recent data demonstrating that these current risk-stratification systems (GOG99, PORTEC2) have difficulty in discriminating between endometrial cancers of low, intermediate and high risk of recurrence [13,16]. It follows that this incomplete prognostication has significant implications on treatment and outcomes: some patients are unnecessarily exposed to the morbidities of adjuvant therapy, while other patients are at increased risk of poor outcome due to lack of adequate treatment [1,6,13,16]. The consequences of inaccurate risk-stratification are especially applicable to the patient population we have studied—stage I, grade 1 endometrioid endometrial cancers—who are stratified into adjuvant therapy versus expectant management following surgery. Recent data produced by TCGA and Talhouk et al. have demonstrated that molecular classification of endometrial cancers is both reproducible and reliably associated with clinical outcomes [12,16]. Therefore, an integrated risk-stratification system utilizing the combination of molecular markers, histopathologic factors and age should be considered in order to achieve more effective prognostication and better outcomes for endometrial cancer patients [6]. Based on our data, *CTNNB1* and MSI-H could be markers included in such an integrated risk-stratification system for stage I, grade 1 endometrioid endometrial cancers. Further evaluation of these markers in a larger cohort would help to confirm their prognostic value.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2019.03.100.

Conflict of interest statement

There were no financial or other forms of outside support provided for this study. There are no potential conflicts of interest to disclose.

Author contribution

Marisa R Moroney, MD contributed project development, IRB proposal, literature review, data abstraction and analysis, abstract and manuscript writing, highlights.

Kurtis D Davies, PhD contributed in molecular testing, and abstract and manuscript editing.

Adam C Wilberger, MD contributed in molecular testing, and abstract and manuscript editing.

Jeanelle Sheeder, MSPH, PhD contributed in data analysis, and abstract and manuscript editing.

Miriam D Post, MD contributed in data collection, molecular testing, and abstract and manuscript editing.

Amber A Berning, MD contributed in data collection, molecular testing, and abstract and manuscript editing.

Christine Fisher, MD contributed in data collection, and abstract and manuscript editing.

Carolyn Lefkowits, MD contributed in data collection, and abstract and manuscript editing.

Saketh R Guntupalli, MD contributed in data collection, and abstract and manuscript editing.

Kian Behbakht, MD contributed in data collection, and abstract and manuscript editing.

Bradley R Corr, MD contributed in project development, IRB proposal, data collection, and abstract and manuscript editing.

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