

Molecular sequencing of serous endometrial carcinoma from patients with or without early recurrence: a pilot study

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Objectives: This study evaluates the mutational burden and molecular profile of stage I serous endometrial carcinoma (SEC) in patients who experienced an early recurrence (within 24 months of surgical staging) compared with those without early recurrence. We hypothesize that differences in the molecular profile impacts the patients' disease-free survival. **Methods:** Cases were identified among patients treated between 2010 and 2017 with a new diagnosis of SEC. The diagnosis was made based on hematoxylin and eosin stained tissue sections and/or immunohistochemical staining consistent with TP53 mutation. Tumors from four patients without early recurrence (followed for 40–62 months) and three patients with early recurrence (17–22 months from staging surgery) were analyzed. Next generation molecular sequencing was performed from paraffin-embedded tissue from each case. The number of mutations, molecular profile, as well as other patient characteristics were compared between the two groups. **Results:** A statistically significantly higher mutational burden among the group without early recurrence and a statistically significantly higher Body mass index among the group with early recurrence was found. A mutation of the SETD2 gene was found only in tumors from patients without recurrence and was not present in any of the tumors from patients with early recurrence. **Conclusion:** This study found a statistically significantly lower mutation burden in SECs from patients who had early recurrence compared to those without early recurrence. It is hypothesized that higher mutational burden favorably changes the prognosis of SEC. We also found an SETD2 mutation only in tumors of patients who had not experienced a recurrence. Further research is needed to confirm these findings.

Keywords

Serous endometrial carcinoma; Endometrial adenocarcinoma; Molecular profile; Mutations

1. Introduction

Endometrial cancer is the most common malignancy of the female genital tract in developed countries. It is the fourth most common cancer in women after breast, lung and colon cancer. There are two subtypes of endometrial cancer: type 1, which includes estrogen-related, endometrioid (grade 1 and 2) carcinomas and type 2, which is not estrogen-related and

includes grade 3 endometrioid and non-endometrioid carcinomas. Endometrioid endometrial carcinoma typically exhibits mutations in PTEN, PIK3CA, K-Ras, and CTNNB1 (beta-catenin) [1]. Non-endometrioid cancers are predominantly serous and clear cell carcinomas and are characterized by having TP53 mutations and chromosomal instability [2].

Serous endometrial carcinoma (SEC) makes up 5–10% of all endometrial carcinomas. Typically, these cases occur in postmenopausal, nonwhite, current smokers who are multiparous and not obese; and these carcinomas behave aggressively [2, 3].

Different mutation burdens occur with different frequencies in endometrial cancers at different stages. A review of the literature shows that TP53 and POLQ mutations are associated with more aggressive cancers [4]. POLE exonuclease domain mutations have been found to carry a favorable prognosis among endometrioid endometrial cancers [5]. There is also evidence that PTEN mutations are more common in endometrioid endometrial cancers, which usually carry a more favorable prognosis [6]. One study found the frequency of PTEN mutation in endometrial cancers to be 84% with frequent overlap with PIK3CA mutations [7]. Specific mutations of the PIK3CA gene have been found in high proportion of metastatic endometrial carcinomas [8–10]. DNA sequencing has been performed on complex atypical hyperplasia tissue sections and it also showed PTEN mutations. However, none of these cases contained both PTEN and PIK3CA mutations. Thus, coexistent PTEN and PIK3CA mutations were significantly associated with endometrioid endometrial carcinoma as compared to complex atypical hyperplasia [11].

In SEC, TP53 mutations are common [12]. Other mutations of SEC have not been well characterized. We hypothesize that the molecular profile of SEC impacts the patient's disease-free survival. This is a pilot study to evaluate the molecular profiles of stage I SECs of patients who experienced early recurrence (less than 24 months) compared with those without early recurrence.

Table 1. Characteristics of cases of stage I serous endometrial carcinoma.

Variable	Without early recurrence	Early recurrence	P-value
	(n = 4)	(n = 3)	
Race/ethnicity (number, %)*			
White	1 (25%)	1 (33%)	0.66
Black	1 (25%)	2 (67%)	
Hispanic	2 (50%)	0 (0%)	
Tobacco use (number, %)*			
Never smoker	3 (75%)	2 (67%)	1
Current smoker	1 (25%)	0 (0%)	
Former smoker	0 (0%)	1 (33%)	
Age (median, range)^	60.5 (42–68)	61.0 (59–72)	0.48
Tumor size (median cm, range)^	5.25 (5–8)	4.5 (1.5–5.5)	0.21
BMI (median kg/m ² , range)^	28.5 (20–35)	50 (46–54)	0.034
Mutated genes (median, range)^	5.5 (4–64)	3 (3–3)	0.028
Mutations/Mb (median, range)^	6.6 (4.8–76.6)	3.6 (3.6–3.6)	0.028

*Categorical variables are shown with number (%) and are compared by Fisher's exact test.

^Continuous variables are shown with median (range) and are compared by two-sample Wilcoxon rank-sum test.

2. Methods

Tumor tissue sections of patients diagnosed with Stage I SEC who have not had cancer recurrence (group 1) were compared to tumor tissue sections of patients diagnosed with Stage I SEC who had early cancer recurrence (group 2), defined as recurrence within 24 months of surgical staging. The Department of Pathology at Temple University Hospital was queried for a list of patients diagnosed with SEC between 2010 and 2017.

We analyzed tissue sections from four patients in group 1 and three patients in group 2. Next generation molecular sequencing was performed at Temple Health Fox Chase Cancer Center Molecular Diagnostic Laboratory from paraffin-embedded tissue from each case. The process of molecular sequencing analyzes DNA from tissue samples. Tissue is placed on a slide and DNA is isolated from it and then the target region amplified. This region is then compared to germline sequences to identify if a mutation exists. Thousands of cells per tissue are used in the analysis. Clinical and demographic data about each patient was collected retrospectively from the electronic health record. Results were then analyzed using the Wilcoxon rank-sum test for continuous variables and the Fisher's Exact test for categorical variables. A *P*-value < 0.05 was considered statistically significant.

3. Results

Patients without recurrence (group 1) had been followed for 40 to 62 months (median 58.5 months). Of the patients in group 2, the recurrence occurred between 17 and 22 months from staging surgery. One cancer recurred in the omentum 17 months after staging surgery, another recurred in the lymph nodes and lungs 20 months after staging surgery, and the third recurred in the right groin lymph nodes 22 months after staging surgery. Of the patients in group 1, three were postmenopausal and one was premenopausal. Of the pa-

tients in group 2, two were postmenopausal and one had not stopped 'menstruating' at the age of 61 years.

The characteristics of the 2 groups are shown on Table 1. There was no statistically significant difference in age (*P* = 0.48), ethnicity (*P* = 0.66), smoking status (*P* = 1), or tumor size (*P* = 0.21) between the two groups. There was statistically significant difference in BMI between the two groups (*P* = 0.034). Number of mutations was compared as number of mutated genes per tumor and number of mutations per million base pairs. A significantly lower number of mutated genes per tumor sample were identified among the early recurrence group (median 3, range 3–3) compared to the group without early recurrence (median 5.5, range 4–64) (*P* = 0.028). A similar finding was noted when comparing mutations per million base pairs between the early recurrence group (*n* = 3), compared to the group without early recurrence (*n* = 4) (median 3.6, range 3.6–3.6 versus median 6.6, range 4.8–76.6, *P* = 0.028).

Table 2. Analysis of select mutations between early and no early recurrence groups.

Mutation	Without early recurrence	Early recurrence	P-value
	(n = 4)	(n = 3)	
TP53			
Present	4 (100%)	2 (67%)	0.43
Not Present	0 (0%)	1 (33%)	
PIK3CA			
Present	3 (75%)	1 (33%)	0.49
Not Present	1 (25%)	2 (67%)	
SETD2			
Present	3 (75%)	0 (0%)	0.14
Not Present	1 (25%)	3 (100%)	

Mutation presence in number of tumors (%), compared by Fisher's Exact test.

Mutations in TP53, PIK3CA, and SETD2 were analyzed and compared between the two groups (Table 2). TP53 mutations were found in 100% of the SEC tissue samples from patients without early recurrence and in 67% of the tissue samples from patients with early recurrence ($P = 0.43$). PIK3CA mutations were found in 75% of the SEC tissue samples from patients without early recurrence and 33% of the tissue samples from patients with early recurrence ($P = 0.49$). SETD2 mutations were found in 75% of the SEC tissue samples from patients without early recurrence and 0% of the tissue samples from patients with early recurrence ($P = 0.14$).

4. Discussion

No statistically significant difference among variables such as race/ethnicity, tobacco use, or age was found among the two groups studied. No statistically significant difference in tumor size was identified. However, this study is limited by the small sample size.

This study found a higher BMI in patients with SEC who experienced an early recurrence compared to those who did not. Obese postmenopausal women have higher circulating estrogen levels compared to non-obese women. The effect of unopposed estrogen on the endometrium is associated with type 1 endometrial cancer but not with type 2. Serous endometrial cancers are not thought to be estrogen-dependent [3]. It is possible that there are factors associated with obesity that contribute to cancer recurrence in patients with SEC. Larger sample size and further investigation are necessary.

This pilot study found a statistically significantly lower number of mutations per tumor among patients with stage I SEC who had early recurrence compared to those who did not. It has been demonstrated in an analysis of cases from the Cancer Genome Atlas that the ultra-mutated group of endometrial tumors showed an increased progression-free survival [1]. It is hypothesized that higher mutational burden favorably changes the prognosis of patients with endometrial cancer.

This study also identified SETD2 mutations only in tumors from patients with stage I SEC without recurrence. SETD2 is known to play an important role in sporadic clear cell renal cell carcinoma and is associated with other cancers such as gliomas [13, 14]. The significance of SETD2 mutations in endometrial carcinoma is an area that needs further research.

5. Conclusions

This pilot study found a statistically significantly lower mutation burden in tumor samples of SEC from patients who had early recurrence compared to those who did not. It is possible that the higher mutational burden allows the immune system to better identify and attack the cancer cells whereas the tumors with a lower mutational burden escape the patient's immune system resulting in an early recurrence. This pilot study also identified a mutation in SETD2 only in tumors from patients without early recurrence. Mutation of

this gene has not been well described in the literature for endometrial cancer and is a subject that needs further investigation. Analysis of a larger sample size is needed to confirm our findings.

Author contributions

MA acquired, analyzed, interpreted the data and drafted the manuscript; ADC helped draft the manuscript. KH provided critical revision of the manuscript for important intellectual content. ST reviewed the histopathology. HZ performed the statistical analysis. EH conceived and designed the study and edited the manuscript.

Ethics approval and consent to participate

This study was deemed exempt from review and requirements for patient informed consent by the institutional review board of Temple University (IRB #25450).

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Conflict of interest

The authors declare no conflict of interest.

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