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Racial differences in oncogene mutations detected in early stage, low grade endometrial cancers

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Abstract

Objective—To describe the pattern and frequency of oncogene mutations in white and African American (AA) women with endometrial cancer, and to determine if racial differences in oncogene mutations exist among women with pathologically similar tumors.

Methods—Endometrial cancer patients from a large, urban hospital were identified through medical records, and representative formalin fixed paraffin embedded tumor blocks were retrieved. The study sample included 150 patients (84 AA) who underwent total abdominal hysterectomy for endometrial cancer. The Sequenom MassARRAY system and the OncoCarta Assay v1.0 (Sequenom), were employed to test for 238 mutations in 19 common oncogenes. Chi-square tests and Fisher's exact tests were used to assess differences in distribution of variables by race and oncogene mutation status.

Results—There were 20 mutations identified in 2 oncogenes (PIK3CA and KRAS) in tumors from 19 women (12.7%). The majority of mutations were found in PIK3CA (16/20). Thirteen percent of endometroid tumors harbored mutations (11 PIK3CA and 2 KRAS), as did 29% of the Malignant Mixed Mullerian tumors (3 PIK3CA and 1 KRAS). There were no observed mutations in serous, clear cell, or mucinous tumor types. Among low grade endometrioid cancers, tumors from AA patients were significantly associated with harboring either a KRAS or PIK3CA mutation (p=0.04), with 7 PIK3CA mutations and all 4 KRAS mutations identified in AA women.

Conclusions—This study provides preliminary evidence that oncogene mutation frequency of some subtypes of histologically similar endometrial carcinoma differ by race. Additional studies are needed to further explore this phenomenon in patients with endometrial carcinoma.

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Introduction

Endometrial cancer is the most common gynecologic cancer, with an estimated 46,470 new cases in the United States in 2011[1]. While the incidence of endometrial cancer is higher among white women, data from the Surveillance, Epidemiology and End Results program suggest the death rate from endometrial cancer in African American (AA) women is nearly twice that of whites (7.2 per 100,000 and 3.9 per 100,000, respectively)[1]. Some of this disparity can be explained by the differences in clinical factors and histopathologic types between the two populations [2]. Type I endometrial cancers are estrogen-related, endometrioid type tumors, which develop in both pre and post-menopausal women, express estrogen and progesterone receptors, and frequently show mutations in DNA-mismatch repair genes. Type I endometrial cancers generally have a good prognosis. Type II endometrial cancers are non-estrogen-related, non-endometrioid type tumors. They predominantly occur in postmenopausal women, do not express hormone receptors, are of high grade and are characterized by p53 mutations. Type II endometrial cancers generally have poor prognosis. AA women are more likely to be diagnosed with Type II endometrial cancers compared to their white counterparts [1, 3].

Stage at diagnosis is also strongly associated with survival, with a 5-year relative survival of 95.8% for women diagnosed at localized stages. For women diagnosed at later stages, 5-year survival is markedly worse, ranging from 67.1% for those diagnosed with regional lymph node spread to 16.2% for those with distant metastatic disease[1]. AA women are diagnosed at later stages and with more aggressive histologic types compared to white women, but still experience poorer survival even after accounting for these factors [4, 5]. Additionally, other research suggests this disparity persists after controlling for co-morbid conditions, access to care, and treatment decisions [6–8]. We hypothesize that there are differences in the molecular biology of tumors from white and AA women, beyond histologic type and grade, which may result in a poorer prognosis.

Mutations in oncogenes have been shown to be key drivers in a variety of cancers, and have also been successfully targeted for treatment [9]. The spectrum of somatic mutations in endometrial cancers have not been well-described, particularly by ethnic group. The goal of this study was to describe the pattern and frequency of oncogene mutations in white and AA women with endometrial carcinoma, and to determine if racial differences in oncogene mutations exist among women with pathologically similar tumors.

Methods

This is a case-only retrospective analysis of patients with endometrial carcinoma who underwent total abdominal hysterectomy for their endometrial carcinoma from 1990–2005 at Henry Ford Hospital, a large urban integrated health system in Detroit, Michigan. All endometrial cancer patients diagnosed and treated at the facility were selected based on the availability of tumor blocks and abstracted medical record data. A total of 266 tumor samples were available for inclusion in the study. Of these, we included all high grade tumors (n=75, 40 from AA and 35 from white women). All remaining tumors from AA women were selected (n=44, low grade), and a random sample of low grade tumors from white women were selected (n=30), one tumor from a white woman with unknown grade was also included, for a final sample of 150 cases (n=66 white, n=84% AA). Demographic and clinical variables were obtained from medical record review. Tumor histologic type, surgical stage, and grade were obtained from the Metropolitan Detroit Cancer Surveillance System registry, part of the SEER program. A gynecologic pathologist (R.A.) confirmed tumor histologic type and grade by slide review. Institutional approval from both institutions was obtained prior to data collection.

Tumor DNA was extracted from formalin fixed paraffin embedded (FFPE) tissue using the QIAamp DNA FFPE Tissue kit (Qiagen, Valencia, CA). The Sequenom Mass ARRAY system and the OncoCarta Assay v1.0 (Sequenom, San Diego, CA), were employed to test for 238 mutations in 19 common oncogenes. A detailed description of the assay's methodology, including a list of detectable mutations has been previously described [10]. Briefly, an initial PCR reaction is performed to amplify a small region of DNA which includes the potential point mutation site. Next, a 10 base DNA oligonucleotide primer binds immediately upstream of the mutation site and is extended by one base into the potential mutation site. The primers are subsequently separated on a matrix-assisted laser desorption/ ionization time of flight (MALDI-TOF) mass spectrometer (Sequenom, San Diego, CA) which is able to quantitatively discern the specific nucleotide that was extended. Manual review of each sample report was completed to confirm the absence or presence of mutations identified by the MassArray Typer Analyzer software (Sequenom, San Diego, CA). Chi-square tests and Fisher's exact tests were used to assess differences in distribution of clinical and demographic variables by race and oncogene mutation status. Differences were considered to be statistically significant at alpha <0.05. All analyses were performed using SAS statistical software, version 9.2 (SAS Institute Inc., Cary, NC).

Results

Table 1 describes demographic and clinical variables by race. Other than BMI (p=0.03), characteristics of the subjects did not vary by race. Of the 70 deaths in the population, 26 were endometrial cancer-related (n=14 white, n=12 black; data not shown). There were 20 mutations identified in 2 oncogenes (PIK3CA and KRAS) in tumors from 19 women (12.7%), shown in Table 2. Thirteen percent of endometroid tumors harbored mutations (11 PIK3CA and 2 KRAS), as did 29% of the Malignant Mixed Mullerian tumors (3 PIK3CA and 1 KRAS). The most common mutations were in the PIK3CA gene, representing 80% (16/20) of the detected mutations. The following mutations were identified: R88Q, in the p85-binding domain, E542K, E545K, M1043I, and P539R in the helical domain, and H1047R in the kinase domain. An additional 4 samples had activating mutations in the KRAS gene (1 G12D, 1 G12V, and 2 Q61H). No other mutations were identified. Only one endometrial cancer-related death occurred in this group, in the woman with the stage IV mixed histology tumor (data not shown).

Overall, there was no difference in proportion of cases having either a PIK3CA or KRAS mutation by race, grade, histologic type, age, smoking history, or BMI (Table 3). Stratified by histologic type, 13% of the endometrioid tumors harbored mutations (11 PIK3CA and 2 KRAS), and 12% of non-endometrioid tumors harbored mutations. Only mixed tumors and malignant mixed Mullerian tumors harbored mutations (3 PIK3CA and 1 KRAS, Table 2), there were no observed mutations in the serous, clear cell, or mucinous tumor types. Among tumors from women with low grade endometrioid cancers, race was significantly associated with harboring either a KRAS or PIK3CA mutation (p=0.04), with all 7 PIK3CA mutations identified in AA women (Table 4). In addition, all 4 KRAS mutations identified were found in specimens from AA women (p=0.13, data not shown).

Discussion

This study provides preliminary evidence that the underlying molecular biology of some subtypes of histologically similar endometrial cancers differ by race. PIK3CA and KRAS mutations were found in endometrial tumors from African American and white women. However, AA women with low grade endometrioid carcinoma of the uterus were more likely to harbor an oncogene mutation than white women. It is notable that no hotspot mutations in the 17 other oncogenes examined were identified in these tumors.

Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases that regulate a variety of pathways involved in carcinogenesis, including cell proliferation, adhesion and survival [11]. PIK3CA codes for the catalytic subunit p110α of PI3K and has been reported to be somatically mutated in a number of cancers [12]. Previous data suggest various mutations in the PIK3CA gene, including mutations found in our samples, induce a gain of PI3K function, with oncogenic potency varying by location [13, 14]. Findings from a phase I study of advanced cancers treated with PI3K/AKT/mTOR inhibitors report the response rate to therapeutic agents targeting this pathway was significantly higher among individuals with PIK3CA mutations (in exons 9 and 20), suggesting that these mutations have clinical predictive value[15].

PIK3CA mutations have been previously described in endometrial cancers. In a series of 45 endometrial serous carcinomas, Hayes et al. reported 15% of tumors harbored mutations [16]. Konstantinova et al. found PIK3CA mutations were more frequent in serous and mixed tumors compared to endometrioid tumors (p-value=0.02), and noted that the location of the mutation differed by pathological characteristics [17]. Similarly, Catasus et al. described location of the PIK3CA mutations varied by histologic type and grade, with exon 20 mutations more common in high-grade carcinomas and in tumors with myometrial invasion[18]. Our study did not identify PIK3CA, or any other mutations, in the 18 serous carcinomas examined, but the tumors with exon 20 mutations were all high grade.

Point mutations in KRAS are relatively common in endometrial cancers, particularly in Type I tumors, but their prognostic significance is unclear [19–23]. These mutations have been found in endometrial hyperplasia, suggesting they are early events in carcinogenesis [23]. Three of the four KRAS mutations identified in our population were in women with stage I disease. All mutations were found in AA women. It is feasible that mutation rates differ by race/ethnicity, with a study by Sasaki et al. finding KRAS mutations in endometrial cancers nearly twice as common among Japanese women compared with white women from the United States [23]. Larger studies are needed to clarify whether racial differences in mutation status exist, and if they are clinically significant.

Our study had limitations that should be noted. First, the Sequenom OncoCarta V1.0 Panel is not specific to all mutations expected in endometrial cancers, and includes only oncogenes, not tumor suppressor genes (such as PTEN or p53, common in endometrioid and serous carcinomas, respectively) or other molecular characteristics that may have prognostic or predictive value. In addition, while many of the activating mutations are found at the hot spots tested for by this panel, it does not detect all of the variation that other methods (i.e. next generation sequencing) could. The Sequenom MassARRAY system does offer a sensitive, high throughput approach that can screen tumors for a large number of mutations in a cost-effective manner, and can detect mutations in a heterogeneous mixture of cancerous and normal tissue 10. Our study population was also limited by sample size, with only 18 serous and one clear cell carcinoma. Some groupings (i.e., endometrioid versus non-endometrioid) may not have strong clinical relevance. Lastly, due to the relatively low proportions of tumors harboring mutations and the excellent endometrial-cancer specific survival of the study population (with only one endometrial cancer-related death among women with tumors harboring mutations), we were unable to examine whether these mutations were significant predictors of prognosis or not.

Conclusions

This study provides preliminary evidence that the underlying molecular biology of some subtypes of histologically similar endometrial cancers differ by race. It is possible that differences in tumor biology are contributing to the disparities in survival seen between

white and AA women with endometrial cancer, similar to the higher incidence of triple negative breast tumors in AA women that contribute substantially to higher mortality rates. Mutation frequency in KRAS, PIK3CA and other genes may differ by race. If these mutations are found to contribute to a more aggressive clinical course in future studies, they may partly explain the poorer survival seen among AA women.

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Table 1

Patient characteristics of the study cohort of 150 endometrial cancers examined for somatic mutations

	White N=66	African American N=84	p value*
Age at Diagnosis			
mean (std)	66.2 (12.3)	63.9 (12.0)	0.26
median (range)	65 (37–88)	65.5 (33–86)	0.40
Smoking Status, N (%)			0.94
Never	47 (71%)	60 (71%)	
Former	7 (11%)	9 (11%)	
Current	10 (15%)	15 (18%)	
Unknown	2 (3%)	0 (0%)	
BMI, N (%)			0.03
<25	13 (20%)	6 (7%)	
25–29.9	15 (23%)	16 (19%)	
30+	34 (52%)	59 (70%)	
Unknown	4 (6%)	3 (4%)	
Modified Charlson Index			0.29
None	36 (55%)	35 (42%)	
1–2	22 (33%)	37 (44%)	
3+	8 (12%)	12 (14%)	
Histology, N (%)			0.15
Endometrioid	45 (68%)	55 (65%)	
Clear cell	1 (2%)	0 (0%)	
Serous	5 (8%)	13 (15%)	
MMMT	8 (12%)	6 (7%)	
Mixed	4 (6%)	10 (12%)	
Mucinous	2 (3%)	0 (0%)	
Unknown	1 (2%)	0 (0%)	
Grade, N (%)			0.45
Low	30 (45%)	44 (52%)	
High	35 (53%)	40 (48%)	
Unknown	1 (2%)	0 (0%)	
FIGO Stage, N (%)			0.12
I	40 (61%)	60 (71%)	
II	5 (8%)	12 (14%)	
III	13 (20%)	8 (10%)	
IV	6 (9%)	4 (5%)	
Unknown	2 (3%)	0 (0%)	
Mutation, N (%)			0.5
No	59 (89%)	72 (86%)	
Yes	7 (11%)	12 (14%)	
Vital Status, N (%)			0.29

	White N=66	African American N=84	p value*
Alive	32 (48%)	48 (57%)	
Deceased	34 (52%)	36 (43%)	

*p value calculations do not include unknown values

Table 2

Clinical characteristics of endometrial tumors harboring somatic oncogene mutations

Gene	Predicted Protein Change	Location	Race	Grade	Histology	FIGO Stage
RAS						
	G12D	Exon 1	AA	High	Endometrioid	I
	G12V	Exon 1	AA	High	MMMT	III
	Q61H	Exon 2	AA	Low	Endometrioid	I
	Q61H	Exon 2	AA	High	Mixed	I
PIK3CA						
	E542K	Exon 9	AA	Low	Endometrioid	II
	E542K	Exon 9	AA	Low	Endometrioid	II
	E542K	Exon 9	White	High	Mixed	IV
	E545K	Exon 9	AA	Low	Endometrioid	I
	E545K	Exon 9	White	High	Endometrioid	I
	H1047R	Exon 20	AA	High	MMMT	I
	H1047R	Exon 20	White	High	MMMT	I
	H1047R	Exon 20	White	High	Endometrioid	I
	M1043I	Exon 20	White	High	Endometrioid	IV
	R88Q	Exon 1	AA	Low	Endometrioid	I
	R88Q	Exon 1	AA	Low	Endometrioid	I
	R88Q	Exon 1	White	High	MMMT	I
	R88Q	Exon 1	AA	High	Endometrioid	I
	R88Q & P539R	Exon 1 Exon 9	White	High	Endometrioid	I
	R38H	Exon 1	AA	Low	Endometrioid	I

Table 3

Demographic and risk factor characteristics of patients with endometrial cancers by somatic mutation status (n=150)

	Mutation (PIK3CA or KRAS)		p value
	Absent, N (%)	**Present, N (%)**	
Age at Diagnosis			
Mean (std)	64.7 (12.2)	66.5 (11.8)	0.54
Median (range)	65 (33–88)	66 (46–84)	0.54
Race			0.5
White	59 (89%)	7 (11%)	
African American	72 (86%)	12 (14%)	
Smoking Status[b]			0.99
Never	93 (87%)	14 (13%)	
Former	14 (88%)	2 (13%)	
Current	22 (88%)	3 (12%)	
BMI[c]			0.45
<25	16 (84%)	3 (16%)	
25–29.9	25 (81%)	6 (19%)	
30+	83 (89%)	10 (11%)	
Modified Charlson Index			0.28
None	59 (83%)	12 (17%)	
1–2	53 (90%)	6 (10%)	
3+	19 (95%)	1 (5%)	
Histology			0.86
Endometrioid	87 (87%)	13 (13%)	
Non-Endometrioid	44 (88%)	6 (12%)	
Grade[a]			0.23
Low	67 (91%)	7 (9%)	
High	63 (84%)	12 (16%)	
FIGO Stage[d]			0.61
I	86 (86%)	14 (14%)	
II	15 (88%)	2 (12%)	
III	20 (95%)	1 (5%)	
IV	8 (80%)	2 (20%)	

Missing values (a-1, b-2, c-7, d-2)

Table 4

Demographic and risk factor characteristics of women with low grade endometrioid endometrial cancers (n=73), by mutation status

	Mutation (PIK3CA or KRAS)		p value
	Absent, N (%)	**Present, N (%)**	
Age at Diagnosis			
Mean (std)	61.6 (12.6)	62.6 (11.5)	0.85
Median (range)	61.5 (33–88)	62 (48–79)	0.88
Race			0.04
White	29 (100%)	0 (0%)	
African American	37 (84%)	7 (16%)	
Smoking Status[a]			0.54
Never	47 (90%)	5 (10%)	
Former	7 (100%)	0 (0%)	
Current	11 (85%)	2 (15%)	
BMI[b]			0.50
<25	7 (100%)	0 (0%)	
25–29.9	10 (83%)	2 (17%)	
30+	47 (90%)	5 (10%)	
Modified Charlson Index			0.52
None	27 (90%)	3 (10%)	
1–2	29 (88%)	4 (12%)	
3+	10 (100%)	0 (0%)	

Missing values (a-1, b-2)