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Cadherins, catenins and cell cycle regulators: Impact on survival in a Gynecologic Oncology Group phase II endometrial cancer trial

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Abstract

Objective—We evaluated the clinical relevance of catenins, cadherins and cell cycle regulators in stage IV or recurrent endometrial carcinoma in a multi-center phase II trial (GOG protocol #119).

Methods—Tissue microarrays of metastatic or recurrent (n=42) tumor were developed and immunohistochemistry performed. Average expression (percent staining × intensity) was assessed in tumor epithelium (^E) and stroma (^S) and categorized into tertiles (T1, T2, T3) for E-cadherin^E, N-cadherin^E, alpha-catenin^E, beta-catenin^E, gamma-catenin^E, p120-catenin^E and Ki-67^E; as negative, below median or above median for p16^E, p27^E and CD44^S; or as negative or positive for p53^E, Ki-67^S and APC^S (adenomatous polyposis coli). End points included response and survival.

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DISCLOSURE/CONFLICT OF INTEREST

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Results—E-cadherin^E, p16^E, and p53^E varied by race (p=0.003, p=0.024, p=0.002,) and N-cadherin^E, Ki-67^E, p16^E and p27^E by tumor type (p=0.015, p=0.011, p=0.005, p=0.021). Correlations were observed among E-cadherin^E with p120^E (r=0.66), p53^E (r=−0.32), alpha-catenin^E (r=0.52), beta-catenin^E (r=0.58), and gamma-catenin^E (r=0.58). High E-cadherin^E (T2 or T3) versus low (T1) expression was associated with better survival in unadjusted (hazard ratio [HR]=0.14, 95% confidence interval [CI]=0.06–0.37 or HR=0.17, 95% CI=0.07–0.42) and adjusted models (HR=0.18, 95% CI=0.05–0.59 or HR=0.22, 95% CI=0.07–0.70). High p16^E versus negative expression was associated with worse survival in unadjusted (HR=3.87, 95% CI=1.74–8.61) and adjusted (HR=4.18, 95% CI=1.28–13.6) models. Positive versus negative expression of p53^E was associated with worse survival in unadjusted (HR=2.31, 95% CI=1.16–4.60) but not adjusted models.

Conclusions—E-cadherin^E and p16^E appear to be clinically relevant, independent prognostic factors in stage IV or recurrent endometrial cancers treated with T+M, and merit further study.

Keywords

endometrial cancer; cadherin; catenin; p16; p120; p53

INTRODUCTION

Endometrial cancer is the most common gynecological malignancy in the United States. The 5-year survival rate is 96% if the cancer is diagnosed at a local stage, but decreases to 17% if diagnosed at an advanced stage [1]. As invasion and metastasis at the time of diagnosis significantly worsen the prognosis, awareness of the biomarkers that may be clinically relevant in endometrial cancer is warranted.

Invasion may be due to reduced cell to cell adhesion properties. Without strong adhesions between cells, cancer cells can more easily, leave their primary location to invade the surrounding tissue, and spread elsewhere [2]. Cadherin is one of the main adhesion molecules in the cell-cell adherens junction. Cadherin molecules are calcium-dependent transmembrane glycoproteins [3]. E-cadherin is the major cadherin in epithelial cells, while N-cadherin is found in neural tissues. The cytoplasmic domain of cadherin binds to a Catenin which can be of the alpha, beta or gamma subtype [2]. Reduced expression of E-cadherin molecules has been associated with many types of cancer and alterations in E-cadherin proteins and their associated cytoplasmic proteins may have a role in determining differentiation in endometrial adenocarcinomas [4, 5].

Biomarkers that play a role in tumor formation by controlling cell cycle proliferation include p16, p27, p53, and Ki-67. p16 functions as a negative regulator of the cell cycle and the association between p16 and HPV is well documented [6–9]. However, p16 overexpression has also been reported in tumors that have no direct link to Human Papilloma Virus (HPV) [10,11]. A cyclin-dependent kinase inhibitor, p27, also functions as a tumor suppressor gene [12]. One of the most commonly mutated tumor suppressor genes in human cancers is p53 [13]. CD44 is a transmembrane cell surface adhesion glycoprotein with important cell-cell and cell-matrix activities [14]. E-cadherin and APC, being components of the Wnt signaling pathway via aberrant DNA methylation may be important in endometrial carcinogenesis [15,16]. The role of cadherin-catenin complexes has been studied in regards to endometrial cancer, but less so in advanced endometrial cancer [17–20]. Understanding correlations and associations between cadherin-catenin proteins and the additional biomarkers listed above has the potential to predict a patient's prognosis and survival, even for women with advanced or recurrent disease.

The aim of this study is to evaluate the differential expression of the proteins of the cadherin-catenin complex in advanced endometrial carcinoma and correlate this with clinical outcomes and survival. We also aim to evaluate any interactions between these complexes and other tumor suppressor genes namely p16, p27, p53, APC, and CD44. We hypothesize that the expression profiles of these biomarkers will influence prognosis and clinical outcome.

MATERIALS AND METHODS

Patients

Between 1991 and 1996, the Gynecologic Oncology Group (GOG) conducted a prospective phase II trial of tamoxifen combined with intermittent medroxyprogesterone acetate. This trial included patients with histologically-confirmed, through central review by the GOG Pathology Committee, advanced, persistent or recurrent endometrial carcinoma considered incurable by local therapy or refractory to local therapy. The disease was to be measurable in 2 dimensions by palpation or imaging. If measurable only by imaging, it was to have a minimum diameter of 3 cm. The tumor must not have received radiation therapy within 3 months prior to entry. Prior therapy with cytotoxic drugs or hormonal therapy was not allowed. Normal hepatic, renal and hematologic functions, the absence of significant infection and GOG performance status 0–2 were required. Patients with past or concomitant malignancy were ineligible. A written informed consent conforming to federal, state and local regulations was obtained from all participants prior to study entry.

Treatment

Patients were treated with weekly cycles of 20 mg oral tamoxifen citrate twice daily. On alternating (even-numbered) weeks, patients also received 100 mg oral medroxyprogesterone acetate twice daily. This treatment regimen is abbreviated T+M.

Clinical End Points and Outcomes

The clinical results of the GOG-119 trial have previously been reported [21]. Progression-free interval (PFI), overall survival (OS) and progression-free survival (PFS) were calculated per standard definitions.

Specimens

The GOG-119 protocol required the submission of frozen metastatic or recurrent tumor removed prior to initiation of T+M treatment. Residual frozen tumor specimens were then formalin-fixed and paraffin-embedded to support biomarker evaluations by immunohistochemistry [22] and these were utilized for the creation of tissue microarrays (TMAs).

Tissue Microarray Array Block and Slide Preparation

TMAs were constructed by selecting regions with viable tumor from the hematoxylin and eosin stained slides. TMA assembly proceeded using a tissue-arraying instrument (Beecher Instrument, Silver Springs, MD). A minimum of 3 tissue cores were taken from each patient's tumor sample. A total of 4 TMA blocks were prepared with 2 of these being duplicates and representing more cores than would have been feasible with 2 TMA blocks alone. Normal controls and navigational aids were also transferred to the recipient block. Multiple 4- μ m sections were prepared. Immunohistochemistry (IHC) assays were run on unstained slides.

Immunohistochemistry Assays

The antibody for E-cadherin (1:100 dilution of mouse monoclonal 36, Transduction Laboratories, Lexington, KY), N-cadherin (1:200 dilution mouse monoclonal 32, Transduction Laboratories), p16 (1:200 dilution mouse monoclonal clone 16, Novocastra Laboratories Ltd., Newcastle Upon Tyne, UK), p27 (1:50 dilution of mouse monoclonal SX53D8 Dako, Carpinteria, CA), p53 (1:200 dilution mouse monoclonal clone DO-7 Dako), p120 (1:200 dilution of mouse monoclonal 15D2, Zymed Laboratories Inc., South San Francisco, CA), alpha-catenin (1:100 mouse monoclonal G-11 Santa Cruz Biotechnology Inc, Santa Cruz, CA), beta-catenin (1:100 dilution of mouse monoclonal clone 14, Transduction Laboratories), gamma-catenin (1:50 dilution mouse monoclonal clone 15, Transduction Laboratories), CD44 (Dako anti-CD44 clone DF1485) and APC (1:50 dilution of rabbit polyclonal C-20; Santa Cruz) was evaluated in conventional sections of formalin-fixed, paraffin-embedded normal endometrium and endometrial carcinoma which served as positive and negative controls. Titration for the appropriate sensitivity and specificity was performed on these tissues. Immunohistochemistry assays were optimized for each biomarker. Briefly, Antigen retrieval was performed in citrate buffer using a Biocare Medical (Walnut Creek, CA) decloaking chamber. TMA slides were incubated with 3% peroxide in absolute methanol, an avidin/biotin blocker, and then with the appropriate primary antibody at 37° C for 1 hour (except p27 1 hour at room temperature), the secondary antibody (Dako Biotinylated Multi-Link anti-mouse, anti-goat, and anti-rabbit immunoglobulin in 40% human serum) at room temperature for 30 minutes. The negative control sections were treated in the same manner, except that primary antibody was replaced with IgG sub class matched immune serum.

Interpretation and scoring of IHC

The TMAs were scored independently by study pathologists (MS, JVR, ZW and AA) who were blinded to clinical information. Consistency for interpretation of stain intensity and percentage cells stained amongst the pathologists was first established by jointly reviewing immunostained examples of TMAs and conventional slide sections of the biomarkers evaluated. At least 2 pathologists reviewed each biomarker section and any discrepancies amongst the reviewers were resolved by re-reviewing the tissue cores across a double headed microscope, before assigning a final scoring. We reported percent positive staining cells (0–100%) and staining intensity (0, 1, 2, and 3). Staining intensities were defined as: 0 = no brown staining at high power, 1 = faint brown staining at high power, 2 = moderate brown staining visible at low power, 3 = dark brown staining at low power. An aggregate score was calculated for each core as percent positive staining × staining intensity (0–300). Mean values, for both tumor epithelium (^E) and stroma (^S), were calculated across all the cores available for a given patient for each biomarker: percent positive staining, staining intensity, and staining index e.g. expression (percent positive staining × staining intensity). Biomarkers were categorized into tertiles (T1, T2, T3) for E-cadherin^E, N-cadherin^E, alpha-catenin^E, beta-catenin^E, gamma-catenin^E, p120-catenin^E and Ki-67^E; as negative, below median or above median for p16^E, p27^E and CD44^S; or as negative or positive for p53^E, Ki-67^S and APC^S.

Statistical Analysis and Methods

Biomarkers were categorized as indicated above. Clinical-pathologic characteristics were compared using Fisher's Exact test, survival probability using the Kaplan-Meier method, and the logrank test to compare groups. Cox proportional hazards models compared groups in unadjusted and adjusted models (with an adjustment for patient age at study enrollment and stratification for performance status and tumor grade). All tests were two-sided, and results considered significant if $p < 0.05$; adjustments were not made for multiple testing as these exploratory analyses were post hoc and are being used to prioritize future testing. Plots

of biomarker pairs and rank correlations helped explore correlations between biomarkers. Statistical analyses were performed using SAS (SAS Institute Inc., Cary NC).

RESULTS

Between June 1991 and February 1996, 61 patients were entered into the phase II GOG 119 study [21] and 60 were eligible. Archival metastatic tumor (N=4) and recurrent tumor (N=38) were available for 42 of the 60 eligible women (70%). All of the 12 biomarkers evaluated were expressed in the tumor epithelium but p53 was the only biomarker that was not expressed in the tumor stroma. Figure 1 shows staining for some of the biomarkers. E-cadherin, N-cadherin, p16, alpha-catenin, beta-catenin, gamma-catenin, CD44, APC, and p120 exhibited cytoplasmic staining. p27, p53, p16, and Ki67 displayed nuclear staining. A few tumor cells also presented with nuclear beta and gamma-catenin. Membrane staining was observed for alpha-catenin, beta-catenin, gamma-catenin, and p120.

Table 1 displays the clinicopathologic characteristics and outcomes for the subset of patients included in the TMAs compared with the full cohort of women who participated in the GOG-119 protocol; their characteristics and outcomes were similar. A majority were over 60 years of age, Caucasian and had symptomatic performance scores and recurrent disease. The most common tumor types were endometrioid and uterine serous cancer. Of the women treated with T+M, >30% experienced either a complete response or partial response (Table 1).

Table 2 displays relationships between categorized expression of the biomarkers and clinicopathologic characteristics. E-cadherin^E, p16^E, and p53^E varied significantly by race. For example, high E-cadherin^E expression was only observed in the Caucasian women whereas p16 and p53 expression were observed in Caucasians and African-Americans. N-cadherin^E, Ki67^E, p16^E, and p27^E were differentially expressed in histologic types. For example, high N-cadherin^E and low Ki-67^E expression were more common in non-endometrioid cancers. Serous adenocarcinomas did not exhibit low p16^E and always expressed some p27^E. None of the biomarkers were associated with tumor grade (Table 2).

Statistically significant correlations were observed among several biomarkers in the tumor epithelium but not in the tumor stroma. For these analyses, average biomarker expression (percent staining × intensity) across replicate cores was examined as continuous rather than categorical variables. Positive correlation were observed between the epithelial expression of E-cadherin^E with p120^E, alpha-catenin^E, beta-catenin^E and gamma-catenin^E; Ki-67^E with p120^E, p27^E and gamma-catenin^E; N-cadherin^E with p120^E and gamma-catenin^E (Table 3). Inverse correlations were observed between E-cadherin^E and p53^E and between Ki-67^E with CD44^E (Table 3).

Women with high E-cadherin^E expression (T2 or T3) versus low expression (T1) had a reduced risk of disease progression and a reduced risk of death, and the association with better survival persisted after an adjustment for patient age at study enrollment and stratification for performance status and tumor grade (Table 4). The Kaplan-Meier plot in Figure 2C also illustrates the survival benefit associated with high versus low E-cadherin^E expression including a 13–18 month difference in median survival time. High p16^E expression (above the median) versus negative expression was associated with similar risk of disease progression, an increased risk of death and worse survival (Table 4, Figures 2A, 2B, 2D). Positive versus negative expression of p53^E was associated with a similar risk of disease progression, an increased risk of death in unadjusted models but not after adjustment for other factors, and worse OS (Table 4, Figures 2A, 2B, 2E). High (T3) versus low (T1) N-cadherin^E and p120^E expression were associated with better OS in unadjusted models but

not after adjustment for other factors, and neither appeared to be associated with PFS (Table 4, Figures 2A–2B). None of the other biomarkers were associated with either PFS or OS (Table 4, Figures 2A–2B and data not shown) or with response.

DISCUSSION

Members of the cadherin-catenin complex play significant roles in cell-cell adhesion and tumor progression in solid tumors including endometrial cancer [4]. The aim of our study was to explore the expression of biomarkers related to cell-cell adhesion and cell cycle in endometrial carcinoma for associations with clinicopathologic characteristics, clinical response, PFS and OS in women with advanced or recurrent disease who participated in a multi-institutional phase II trial of T+M conducted by the GOG [21].

Biomarker Associations with Clinicopathologic Characteristics

Our study showed that high E-cadherin^E expression was only observed in the Caucasian women whereas p16^E and p53^E expression were observed both in Caucasians and African-Americans. In addition, high p16^E expression and any p53^E expression were both more common in African-American women compared with Caucasians. In contrast, Schimp et al. and Lukes et al., showed that p53 expression did not vary significantly by race [23,24]. Differences in samples size, evaluation criteria for the individual biomarkers and the inclusion versus exclusion of recurrent tumors may contribute to the disparities observed between studies.

High p16 expression was identified more often in serous carcinomas than in endometrioid or other types. This is supported by Engelson et al, who found p16 expression to correlate with serous/clear cell histologic subtypes [25]. We also showed that uterine serous adenocarcinomas expressed at least some p27^E. However, Nycum et al did not find any association between p27 expression and histologic type of endometrial cancer [26]. The observation that high N-cadherin^E was more common in non-endometrioid cancers is an intriguing, novel finding considering that N-cadherin has been described to originate in neural tissues and merits further study [2].

None of our biomarkers were associated with tumor grade, unlike some other reports [18–20]. We did not observe any evidence to suggest that any of the biomarkers was associated with grade even when the serous papillary tumors were excluded from the analysis. Associations with grade may require larger sample sizes to detect the relationship. Alternatively, inclusion of low and intermediate risk patients with stage I, II or III disease may be required to detect associations between these biomarkers and grade.

Associations between Biomarkers

We report many positive and negative correlations between the biomarkers evaluated. Others have shown correlations between the expression of p27 and Ki-67 [27] and absent/low expression of p27 and absent/low expression of p53 [12]. These interactions have the potential to shed light on the pathobiology of endometrial cancer.

Biomarker Associations with Survival

Increased E-cadherin expression was associated with longer median survival and reduced risks of disease progression and death. Removal of the serous cancers from the Cox regression analysis did not alter the association between E-cadherin and PFS or survival (data not shown). Our findings support the hypothesis that cell adhesion molecules when expressed in tumor cells will influence prognosis and outcomes, and are in agreement with those of Mell et al. [28]. It is important to note that the patients in our study had stage IV or

recurrent endometrial carcinoma and in theirs had stage I-III patients. Combined results from both studies would suggest that E-cadherin expression is associated with a reduced risk of progression and death in all stages of endometrial cancer (I-IV) as was also reported by Kim et al. [29]. Our results are also in line with those of Scholten et al. who report that in a univariate analysis, 5 and 10-year survival rates were 88% each for patients with E-cadherin positive tumors, while the survival rates were 79% and 71% for patients with negative E-cadherin expression [18].

We report that high versus negative expression of p16 in the tumor epithelium was associated with worse survival and an increased risk of death, and appeared to have independent prognostic value. These findings are supported by those of Engelsen et al [25]. The 5-year survival rate for patients with normal expression of p16 was 85%, but decreased to 50% among patients with increased p16 expression. p16 is used as a surrogate for HPV and for differentiating adenocarcinomas of endocervical origin from those of endometrial origin [30]. Our results, and those of Engleson et al., raise questions about the validity of using p16 for this role since endometrial carcinomas did express p16 in our hands and may serve as an indicator of bad prognosis [25]. Ignatov et al., did not find a correlation between cancer-specific 5-year survival and p16 expression [31]. This remained true even when they used different cut off values for p16 expression in cells (15%, 25%, or 35%). However, they did not take intensity into consideration and the differences between the studies may possibly be explained by this. Expression of p53 in the tumor epithelium was associated with a worse survival but was not an independent prognostic factor. This is concordant with the results of Engelsen et al [25]. The 5-year survival rates dropped from 85% with normal expression of p53, to 52% in patients with pathologic expression. p53 is one of the few IHC stains in addition to ER and progesterone receptor (PR) that is utilized in endometrial cancer pathology work ups, therefore its correlation with decreased survival lends it additional significance as a prognostic indicator. We have previously reported in this patient population that ER was associated with objective clinical response to treatment with T+M [22]. However, none of the biomarkers in the current study were associated with response.

We did not find a significant relationship between Ki-67 and PFS or OS. This is supported by Kallakury et al. [32]. Some report that the S-phase fraction of Ki-67 has a stronger prognostic value [33–36]. With respect to α - and β -catenin, Scholten et al.'s findings, deviate from ours, in that they found their expression to correlate with improved cancer specific survival [18]. However, they did not include Stage IV cases, while we have [18]. A significant relationship was not observed between the expression of CD44 and PFS or OS. While our analysis focused on standard CD44, the subject of many has been the splice variants (isoforms), especially CD44 v6) and has yielded contradictory trends, but falling short of statistical significance [37–41].

Study Limitations and Conclusions

The sample size is a limitation that should be addressed in future investigations. In addition, these results pertain to a specific clinical and treatment scenario and caution should be exercised in extrapolating this information to all groups of endometrial carcinoma. In conclusion, E-cadherin and p16 appear to be clinically relevant tumor biomarkers with independent prognostic value in stage IV or recurrent endometrial cancer patients treated with T+M.

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Highlights

High E-cadherin expression is associated with better survival

High p16 expression is associated with worse survival

E-cadherin and p16: independent prognostic factors in advanced endometrial cancers

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Figure 1.

Immunohistochemistry photomicrographs for select markers in the tumor epithelium or stroma of representative endometrial carcinomas in the GOG#119 tissue microarrays (TMAs). Representative expression of low E-cadherin^E (T1-lowest tertile) [A] compared with high E-cadherin^E (T3-highest tertile) [B]. The inset shows a cross section of the TMA core of (A). Representative endometrial carcinoma exhibiting no N-cadherin expression (negative) [C] compared with high N-cadherin^E expression [D]. The inset shows that stromal elements are negative for N-cadherin [D]. Representative endometrial carcinoma displaying no p16 expression (negative) [E] compared with high p16^E expression in the nucleus and cytoplasm of representative carcinoma cells [F]. The inset shows a cross section

of the TMA core in panel F and displays that expression is confined to tumor and non-tumor tissue (bottom of the TMA) is negative for p16.

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A.

PFS, Adjusted Model

B.

Survival, Adjusted Model

C.

D.

E.

Figure 2.

Forest plots for catenins, cadherins and cell cycle regulators in tumor epithelium (^E) and stroma (^S) for progression-free survival [A] or overall survival [B] with hazard ratios (HR) and 95% confidence intervals (CI) based on Cox models with an adjustment for patient age at study enrollment and stratification for performance status and tumor grade. When the 95% CI does not overlaps a HR of 1.0 (center line), the association was significant. Biomarkers were categorized into tertiles (T1-lowest tertile, T2-middle tertile, T3-highest tertile) for E-cadherin^E, N-cadherin^E, alpha-catenin^E, beta-catenin^E, gamma-catenin^E, p120-catenin^E and Ki-67^E; as negative, below median or above median for p16^E, p27^E and CD44^S; or as negative or positive for p53^E, Ki-67^S and APC^S. Kaplan-Meier survival distributions provided for tumor epithelial expression of E-cadherin^E [C], p16^E [D], or p53^E [E]. Logrank test was used to test the equality in the survival distributions [C–E]. Significant associations (p<0.05) with survival were observed for E-cadherin^E [C], p16^E [D], or p53^E [E]. Death is the event and censored indicates patients who were alive at last contact [C–E]. Median survival times are provided in months with 95% CI.

Table 1

Clinicopathologic characteristics and outcomes.

	TMA Cases		All GOG-119 Cases	
	Cases	%	Cases	%
Age				
<=60	11	26.2	15	25.0
61–70	16	38.1	22	36.7
71–80	9	21.4	16	26.7
>=81	6	14.3	7	11.7
Race				
Caucasian	34	81.0	50	83.3
African American	7	16.7	8	13.3
Other	1	2.4	2	3.3
Performance Status				
0 – asymptomatic	19	45.2	26	43.3
1 – symptomatic	18	42.9	26	43.3
2 – symptomatic	5	11.9	8	13.3
Stage				
IV	4	9.5	6	10.0
Recurrent	38	90.5	54	90.0
Histologic Type				
Endometrioid	23	54.8	32	53.3
Serous adenocarcinoma	9	21.4	14	23.3
Adenocarcinoma, not specified	4	9.5	6	10.0
Mixed epithelial	2	4.8	3	5.0
Adenosquamous	2	4.8	3	5.0
Squamous cell carcinoma	1	2.4	1	1.7
Villoglandular adenocarcinoma	1	2.4	1	1.7
Tumor Grade				
1	12	28.6	15	25.0
2	15	35.7	17	28.3
3	15	35.7	27	45.0
Not Specified			1	1.7
Prior Radiotherapy				
No	17	40.5	24	40.0
Yes	25	59.5	36	60.0
Tumor Responses				
Complete Response (CR)	3	7.1	6	10.0
Partial Response (PR)	11	26.2	13	21.7

	TMA Cases		All GOG-119 Cases	
	Cases	%	Cases	%
Stable Disease (SD)	15	35.7	20	33.3
Increasing Disease (ID)	10	23.8	16	26.7
Not evaluable	3	7.1	5	8.3
Total	42	100.0	60	100.0

Associations between biomarker expression and clinicopathologic characteristics in epithelial (E) and stromal (S) elements of metastatic or recurrent endometrial cancers.

Table 2

Biomarker	Clinicopathologic Characteristics											
	Race (%)				Histologic Type (%)				Tumor Grade (%)			
	n	Caus (n=34)	AA (n=7)	Oth (n=1)	p	End (n=23)	Ser (n=9)	Oth (n=10)	p	G1 (n=12)	G2 (n=15)	G3 (n=15)
E-cadherin ^E												
T1: Low	14	21	86	100	0.003	39	44	10	0.061	33	27	40
T2: Moderate	14	38	14	0		17	33	70		25	40	33
T3: High	14	41	0	0		43	22	20		42	33	27
N-cadherin ^E												
T1: Low	14	32	29	100	1.000	35	33	30	0.015	33	20	47
T2: Moderate	14	32	43	0		52	11	10		33	53	13
T3: High	14	35	29	0		13	56	60		33	27	40
p120-catenin ^E												
T1: Low	14	26	57	100	0.085	35	33	30	0.969	42	27	33
T2: Moderate	14	32	43	0		30	44	30		33	33	33
T3: High	14	41	0	0		35	22	40		25	40	33
alpha-catenin ^E												
T1: Low	14	32	43	0	1.000	26	22	60	0.403	42	33	27
T2: Moderate	14	32	29	100		39	33	20		33	33	33
T3: High	14	35	29	0		35	44	20		25	33	40
beta-catenin ^E												
T1: Low	14	35	29	0	0.256	22	44	50	0.556	33	20	47
T2: Moderate	14	26	57	100		39	22	30		33	47	20
T3: High	14	38	14	0		39	33	20		33	33	33
gamma-catenin ^E												

Biomarker	Clinicopathologic Characteristics												
	Race (%)					Histologic Type (%)					Tumor Grade (%)		
	n	Caus (n=34)	AA (n=7)	Oth (n=1)	p	End (n=23)	Ser (n=9)	Oth (n=10)	p	G1 (n=12)	G2 (n=15)	G3 (n=15)	p
T1: Low	14	26	57	100	0.256	39	22	30	0.199	42	33	27	0.814
T2: Moderate	14	38	14	0		35	11	50		33	40	27	
T3: High	14	35	29	0		26	67	20		25	27	47	
Ki67 ^E													
T1: Low	14	32	43	0	0.665	17	44	60	0.011	33	27	40	0.814
T2: Moderate	14	35	14	100		48	0	30		25	47	27	
T3: High	14	32	43	0		35	56	10		42	27	33	
p16 ^E													
Negative	21	56	14	100	0.024	57	22	60	0.005	58	53	40	0.120
Below median: low	10	26	14	0		30	0	30		17	40	13	
Above median: high	11	18	71	0		13	78	10		25	7	47	
p27 ^E													
Negative	19	47	43	0	0.552	57	0	60	0.021	50	67	20	0.144
Below median: low	11	26	14	100		22	44	20		25	13	40	
Above median: high	12	26	43	0		22	56	20		25	20	40	
p53 ^E													
Negative	29	79	14	100	0.002	78	33	80	0.052	58	87	60	0.196
Positive	13	21	86	0		22	67	20		42	13	40	
Ki-67 ^S													
Negative	24	56	71	0	0.410	57	56	60	1.000	75	40	60	0.209
Positive	18	44	29	100		43	44	40		25	60	40	
APC ^S													
Negative	30	74	71	0	0.404	65	67	90	0.346	92	60	67	0.172
Positive	12	26	29	100		35	33	10		8	40	33	

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Biomarker	Clinicopathologic Characteristics												
	n	Race (%)			p	Histologic Type (%)				p	Tumor Grade (%)		
		Caus (n=34)	AA (n=7)	Oth (n=1)		End (n=23)	Ser (n=9)	Oth (n=10)	G1 (n=12)		G2 (n=15)	G3 (n=15)	
CD44 ^s													
Negative	7	18	14	0	0.834	17	11	20	0.987	25	13	13	0.834
Below median: low	17	38	57	0		43	44	30		42	33	47	
Above median: high	18	44	29	100		39	44	50		33	53	40	

Categorized biomarker expression (percent staining × intensity).

N: cases, %; column percentage (e.g., high E-cadherin was observed in 41% of Caucasians and none of the African-American or other racial groups, respectively), Caus: Caucasian, AA: African American, Oth: Other, End: Endometrioid, Ser: Serous, G1: well differentiated, G2 moderately differentiated, G3: poorly differentiated or not graded.

p<0.05 are bolded

Table 3

Rank correlation coefficients between marker expression in tumor epithelium above the diagonal and in stroma below the diagonal.

Tumor Epithelium (E)													
	APC	CD44	E-cad	Ki67	N-cad	P120	P16	P27	P53	α -cat	β -cat	γ -cat	
Tumor Stroma (S)	APC	0.23	-0.14	0.03	0.19	-0.01	0.01	0.17	0.08	0.09	-0.21	0.21	
	CD44	0.17	-0.04	-0.33*	-0.10	0.08	-0.20	0.06	0.00	-0.24	-0.19	-0.03	
	E-cad	-0.17	0.14	0.17	0.16	0.66*	-0.16	-0.19	-0.32*	0.52*	0.58*	0.58*	
	Ki67	0.02	-0.04	0.46	0.15	0.30*	0.07	0.33*	0.23	0.70	0.62	0.36*	
	N-cad	0.46	0.07	-0.09	0.08	0.44*	0.24	0.05	0.10	0.27	0.26	0.32*	
	P120	0.17	0.04	0.25	0.15	0.21	-0.04	-0.10	-0.21	0.54	0.71	0.65	
	P16	-0.13	0.18	0.00	0.03	-0.06	-0.13	0.09	0.57	0.04	0.07	0.09	
	P27	0.04	0.04	0.26	0.35	0.11	0.18	-0.21	0.18	0.22	0.06	0.08	
	P53	NE	NE	NE	NE	NE	NE	NE	NE	-0.06	-0.09	-0.12	
	α -cat	0.04	-0.04	0.30	0.05	0.01	-0.13	0.19	-0.08	NE	0.77	0.68	
β -cat	0.21	0.02	0.03	0.09	0.47	-0.04	0.22	0.25	NE	0.39	0.51		
γ -cat	-0.10	0.05	0.27	0.15	0.15	0.44	-0.06	0.31	NE	-0.06	-0.10		

NE: not evaluable, E-cad: E-cadherin, N-cad: N-cadherin, α -cat: alpha-catenin, β -cat: beta-catenin, γ -cat: gamma-catenin

* $p < 0.05$

Negative correlations indicate an inverse relationship.

Associations between biomarker expression and clinical outcome in epithelial (E) and stromal (S) elements of metastatic or recurrent endometrial cancers.

Table 4

Biomarker	Progression-Free Survival						Overall Survival					
	Unadjusted Model			Adjusted Model			Unadjusted Model			Adjusted Model		
	HR	(95% CI)	p	HR	(95% CI)	p	HR	(95% CI)	p	HR	(95% CI)	p
E-cadherin ^E												
T1: Low	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
T2: Moderate	0.34	(0.15, 0.76)	0.008	0.30	(0.10, 0.86)	0.025	0.14	(0.06, 0.37)	< 0.001	0.18	(0.05, 0.59)	0.004
T3: High	0.32	(0.14, 0.73)	0.006	0.44	(0.16, 1.21)	0.112	0.17	(0.07, 0.42)	< 0.001	0.22	(0.07, 0.70)	0.010
N-cadherin ^E												
T1: Low	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
T2: Moderate	0.48	(0.21, 1.08)	0.076	0.60	(0.22, 1.64)	0.316	0.54	(0.25, 1.18)	0.122	0.65	(0.24, 1.80)	0.408
T3: High	0.63	(0.29, 1.36)	0.236	0.90	(0.37, 2.16)	0.807	0.33	(0.14, 0.75)	0.009	0.43	(0.16, 1.17)	0.097
p120-catenin ^E												
T1: Low	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
T2: Moderate	0.67	(0.31, 1.44)	0.302	1.05	(0.41, 2.69)	0.913	0.54	(0.24, 1.20)	0.129	0.47	(0.16, 1.36)	0.161
T3: High	0.76	(0.35, 1.64)	0.482	1.05	(0.36, 3.11)	0.928	0.44	(0.20, 0.98)	0.045	0.39	(0.13, 1.18)	0.096
alpha-catenin ^E												
T1: Low	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
T2: Moderate	1.47	(0.68, 3.18)	0.330	1.31	(0.49, 3.52)	0.587	1.27	(0.56, 2.89)	0.575	0.88	(0.34, 2.26)	0.790
T3: High	0.88	(0.42, 1.86)	0.738	0.69	(0.22, 2.16)	0.523	0.99	(0.45, 2.20)	0.981	0.54	(0.18, 1.63)	0.274
beta-catenin ^E												
T1: Low	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
T2: Moderate	1.03	(0.48, 2.19)	0.946	0.74	(0.28, 1.98)	0.549	1.37	(0.62, 3.02)	0.442	1.41	(0.50, 3.95)	0.511
T3: High	0.69	(0.32, 1.48)	0.342	0.60	(0.22, 1.67)	0.329	0.67	(0.30, 1.50)	0.333	0.48	(0.16, 1.41)	0.181
gamma-catenin ^E												
T1: Low	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA

Biomarker	Progression-Free Survival						Overall Survival					
	Unadjusted Model			Adjusted Model			Unadjusted Model			Adjusted Model		
	HR	(95% CI)	P	HR	(95% CI)	P	HR	(95% CI)	P	HR	(95% CI)	P
T2: Moderate	0.77	(0.35, 1.70)	0.519	1.24	(0.48, 3.21)	0.659	0.53	(0.23, 1.20)	0.128	0.48	(0.19, 1.25)	0.134
T3: High	0.55	(0.26, 1.19)	0.131	0.46	(0.17, 1.24)	0.125	0.59	(0.27, 1.28)	0.182	0.36	(0.12, 1.03)	0.057
Ki67 ^E												
T1: Low	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
T2: Moderate	0.82	(0.38, 1.76)	0.615	0.81	(0.33, 2.01)	0.649	0.65	(0.29, 1.46)	0.294	0.48	(0.18, 1.30)	0.149
T3: High	1.07	(0.51, 2.27)	0.859	0.77	(0.28, 2.14)	0.618	1.06	(0.49, 2.32)	0.883	0.59	(0.22, 1.59)	0.299
p16 ^E												
Negative	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
Below median: low	0.83	(0.38, 1.80)	0.636	0.34	(0.08, 1.36)	0.125	0.99	(0.44, 2.22)	0.975	0.39	(0.09, 1.62)	0.196
Above median: high	1.54	(0.73, 3.27)	0.258	2.02	(0.67, 6.05)	0.209	3.87	(1.74, 8.61)	<0.001	4.18	(1.28, 13.6)	0.018
p27 ^E												
Negative	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
Below median: low	1.12	(0.53, 2.39)	0.766	0.54	(0.17, 1.79)	0.316	1.70	(0.79, 3.70)	0.177	0.92	(0.29, 2.95)	0.893
Above median: high	0.64	(0.30, 1.37)	0.248	0.35	(0.12, 1.04)	0.058	0.68	(0.30, 1.54)	0.350	0.51	(0.18, 1.41)	0.191
p53 ^E												
Negative	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
Positive	1.28	(0.66, 2.50)	0.461	1.28	(0.53, 3.07)	0.584	2.31	(1.16, 4.60)	0.018*	1.99	(0.78, 5.07)	0.150
Ki-67 ^S												
Negative	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
Positive	1.35	(0.72, 2.55)	0.349	0.87	(0.39, 1.91)	0.723	1.03	(0.54, 1.95)	0.934	0.97	(0.41, 2.30)	0.947
APC ^S												
Negative	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
Positive	1.97	(0.97, 4.00)	0.060	1.92	(0.69, 5.28)	0.210	1.41	(0.70, 2.80)	0.334	1.65	(0.70, 3.90)	0.254
CD44 ^S												

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Biomarker	Progression-Free Survival						Overall Survival					
	Unadjusted Model			Adjusted Model			Unadjusted Model			Adjusted Model		
	HR	(95% CI)	p	HR	(95% CI)	p	HR	(95% CI)	p	HR	(95% CI)	p
Negative	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA	1.00	NA	NA
Below median: low	1.55	(0.61, 3.96)	0.356	1.26	(0.33, 4.81)	0.739	1.13	(0.43, 2.96)	0.809	0.91	(0.23, 3.51)	0.886
Above median: high	2.23	(0.85, 5.85)	0.105	1.33	(0.36, 5.00)	0.670	1.22	(0.48, 3.11)	0.681	1.25	(0.32, 4.88)	0.749

Categorized biomarker expression (percent staining × intensity).

NA: not applicable

Estimated hazard ratio (HR) and 95% confidence interval (95% CI) for Cox regression analysis.

[†] with an adjustment for patient age at study enrollment and stratification for performance status and tumor grade.

When the 95% CI does not overlap a HR of 1.0, the association was significant (p<0.05 are bolded).