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Original contribution

Keratin 17 is a negative prognostic biomarker in high-grade endometrial carcinomas☆,☆☆,★

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Summary Keratin 17 (K17) has been established as a negative prognostic biomarker in cervical and ovarian cancers but has not previously been evaluated as a prognostic biomarker in endometrial adenocarcinoma. The association of K17 with decreased patient survival may be explained in part by the discovery that K17 drives tumor aggression by serving as a nuclear shuttle of p27, leading to cell cycle progression and tumor growth. The current study tests the hypothesis that K17 mRNA and protein levels correlate with decreased survival of patients with high-grade endometrial cancer. Gene expression data (mRNA) from The Cancer Genome Atlas were analyzed for 271 high-grade endometrial carcinomas and K17 immunohistochemistry (IHC) was performed on a separate cohort of 119 high-grade endometrial cancer cases from two academic medical centers. Survival analyses were determined by Cox proportional hazards regression. High K17 mRNA and IHC correlated with decreased overall survival (HR: 1.8, $P = .0101$, HR: 1.8, $P = .0488$, respectively). K17 was positive in malignant glandular cells of the endometrium but not in other tissues, including endometrial stroma, myometrium and uterine sarcoma. These results support the conclusion that K17 is a negative prognostic biomarker in high-grade endometrial carcinoma and that K17 IHC test results could be used to inform decisions related to therapeutic intervention.

☆ **Competing interests:** Stony Brook University and the co-senior authors (K.R.S. and L.E.H.) have licensed K17 as a biomarker for cervical and urothelial carcinomas but there is no conflict of interest with the current study.

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1. Introduction

There is an unmet need to identify prognostic markers in high-grade endometrial adenocarcinoma that could provide information to help guide treatment and to provide more accurate predictions of patient outcomes. In the United States, endometrial cancer is the most common gynecologic malignancy [1]. Low-grade endometrial cancers are often caused by estrogen excess and account for approximately 80% of all uterine cancers. Patients with these low-grade tumors have a favorable prognosis. High-grade tumors, in contrast, are estrogen-independent and often have a poor prognosis [2-4]. While disease stage and histology largely dictate prognosis, patients with similar stage and grade can vary greatly in their survival outcomes [5]. The current standard-of-care treatment for patients with endometrial cancer is hysterectomy, with or without adjuvant chemotherapy. Categorizing patients based on disease aggression can help clinicians make more insightful decisions regarding the appropriate treatment option. Patients with aggressive disease should be closely monitored while patients with more indolent disease should be spared of unnecessary tests or interventions. Therefore, there remains a need to identify novel prognostic biomarkers in endometrial adenocarcinoma.

One potential candidate is keratin 17 (K17), which has been demonstrated to be a powerful negative prognostic biomarker in several cancers, including other tumors of the female genital tract (cervical squamous cell carcinoma, endocervical adenocarcinoma, and surface epithelial-derived ovarian carcinomas), triple negative breast cancer, gastric adenocarcinoma, oropharyngeal squamous cell carcinoma, and pancreatic adenocarcinoma [6-12]. K17 is an intermediate filament protein that is normally expressed in the ectodermal germ layer during embryogenesis but is silenced in most adult somatic tissues [13]. Mechanistic studies demonstrated that in response to mitogenic signaling, K17 translocates into the nucleus via a nuclear localization signal, where it binds to p27, a G_1-S cell cycle inhibitor. The p27-K17 complex is then exported into the cytoplasm, where p27 is degraded, leading to sustained cell cycle progression [14]. Additionally, in vivo murine models showed that the absence of K17 inhibited the progression of cervical dysplasia and tumor growth, further supporting our previous findings that high expression of K17 is prognostic for reduced survival in cervical cancer [14,15]. These observations prompted our current study to test the hypothesis that higher levels of K17 mRNA and immunohistochemical staining for K17 are associated with decreased survival of patients with high-grade endometrial carcinoma.

2. Materials and methods

2.1. TCGA data mining of K17 mRNA

mRNA expression data were obtained from TCGA (The Cancer Genome Atlas), based on RNA Sequencing (RNAseq) analyses of bulk tumor from patients that underwent hysterectomy for endometrial cancer from 2002 to 2013 (Table 1). mRNA data from 271 cases high-grade endometrial cancers, including 112 grade 3 endometrioid, 93 papillary serous, 51 carcinosarcomas, and 15 mixed or unknown subtype cases were downloaded from cBioPortal.org [16,17]. Overall survival time was calculated from the initial pathologic diagnosis date until either death or the last communication contact with the patient. Staging was based on the FIGO staging system [18].

To compare the relationship between K17 mRNA status (high versus low) and TCGA molecular subgroups of endometrial cancer, data from 81 cases were selected from the original 373 from the TCGA publication [19]. Selection criteria were (1) availability of K17 mRNA levels and (2) high-grade endoemetrial cancer cases. Eight cases of DNA polymerase epsilon catalytic unit mutation (POLE), 23 cases of microsatellite instability (MSI), 5 cases of copy-number low (CN Low), and 45 cases of copy-number high (CN high) were used for comparison.

2.2. Case selection

Formalin-fixed, paraffin-embedded (FFPE) remnant tissue blocks of 119 hysterectomies of high-grade endometrial carcinomas from January 2002 to January 2018 were selected from Stony Brook Medicine and University of Massachusetts Memorial Medical Cancer Registries. These included five grade 3 endometrioid carcinomas, 62 papillary serous carcinomas, and 52 carcinosarcomas. This cohort was constructed to provide a roughly equivalent number of high-grade cases of each major histopathologic type. Cases were selected consecutively within each subtype. Overall survival was calculated from the initial date of histologic diagnosis until either death or the last date of clinical contact with the patient. Variables included in this analysis were tumor stage which were based on FIGO systems of classification [18]. This data was obtained from the pathology reports for each specimen. For three cases where histological grade was not recorded in the database, tumor grade was assigned based on review of the prior endometrial biopsies (2 cases) or an hematoxylin and eosin (H&E) stained slide from the block that was processed for IHC. Analyses incorporating lymph node status, metastasis, and chemotherapy treatment were not included due to lack of power. Data regarding disease status (cancer-free, never cancer-free, or cancer recurrence) were available for 92% (109/119) of the cases, including 100% (5/5) of grade 3 endometrioid carcinomas, 95% (59/62) of papillary serous carcinomas, and 87% (45/52) of carcinosarcomas. All studies were conducted under approval of the Stony Brook Medicine and University of Massachusetts Institutional Review Boards.

To explore K17 expression in other maligiant uterine cancers, we selected cases of clear cell carcinoma (n = 41), low-grade (type 1) endometrioid carcinomas (n = 82), endometrial stromal sarcoma (n = 9), and leiomyosarcoma (n = 13) from the Stony Brook Medicine and UMass Memorial Cancer

Table 1 Baseline characteristics of patients diagnosed with high-grade endometrial carcinoma for keratin 17 (K17) gene expression (mRNA) and immunohistochemistry (IHC) datasets

	Cohort 1: K17 mRNA [a]				Cohort 2: K17 IHC [b]			
	Censored [c] (n = 193)	Failure [d] (n = 78)	Total (n = 271)	*P*	Censored [c] (n = 62)	Failure [d] (n = 57)	Total (n = 119)	*P*
Age at diagnosis, mean ± SD	66.32 ± 10.34	67.74 ± 11.10	66.73 ± 10.56	.3166 [h]	67.68 ± 8.719	66.02 ± 11.15	66.88 ± 9.947	.3654 [h]
Survival (mo), mean ± SD	34.16 ± 26.39	24.94 ± 19.86	31.51 ± 25.00	.0020 [i]	41.06 ± 32.00	26.89 ± 30.42	34.27 ± 31.92	.0149 [h]
Clinical stage, n (%) [e]								
I/II	135 (84)	25 (16)	160 (59)	<.0001 [j]	41 (65)	22 (35)	63 (53)	.0078 [j]
III/IV	58 (53)	52 (47)	110 (41)		20 (39)	31 (61)	51 (43)	
N/A [f]	0 (0)	1 (100)	1 (0.3)		1 (20)	4 (80)	5 (4)	
Histological type, n (%)								
Endometrioid, grade 3	95 (85)	17 (15)	112 (41)	<.0001 [j]	3 (50)	2 (50)	5 (5)	.0034 [j]
Serous	66 (71)	27 (29)	93 (34)		41 (66)	21 (34)	62 (52)	
Carcinosarcoma	21 (41)	30 (59)	51 (19)		18 (35)	34 (65)	52 (44)	
N/A [g]	11 (73)	4 (27)	15 (6)					
K17 status, n (%)								
Low	124 (76)	40 (24)	164 (61)	.0551 [k]	47 (56)	37 (44)	84 (71)	.2293 [k]
High	69 (64)	38 (36)	107 (39)		15 (43)	20 (57)	35 (29)	

Abbreviations: K17, keratin 17; N/A, not available.

[a] Gene expression data were obtained from The Cancer Genome Atlas (TCGA).

[b] Endometrial cancer tissues were obtained from the Stony Brook (SB) Medicine Cancer Registry and the UMass Memorial Cancer Registry.

[c] Patient's last known status is alive based on communications.

[d] Death due to primary (endometrial) carcinoma.

[e] Stage I/II: local disease; III/IV: metastatic disease.

[f] Clinical stage was unknown for 1 case from the TCGA database. Clinical stage was unknown for 5 cases from the Stony Brook Medicine Cancer Registry; patients had hysterectomy prior to 1998 and original pathology reports were not available.

[g] Cases with a mix of two or more carcinomatous histological subtypes.

[h] Student's *t* test (unpaired).

[i] Mann-Whitney *U* test for unequal variances.

[j] χ^2 test.

[k] Fisher's exact test.

Registries. We also explored K17 expression in benign endometrial tissues from patients who underwent hysterectomies with no history of uterine neoplasia between 2008 and 2010, and identified cases of leiomyoma (n = 9), atrophic endometrium (n = 18), secretory endometrium (n = 12) and proliferative endometrium (n = 11).

2.3. Immunohistochemical studies

Immunohistochemistry (IHC) for K17 was performed as previously reported [9-11,14]. Briefly, 5 μm tissue sections were deparaffined with xylene and rehydrated using graded alcohols, antigen retrieval was performed in a decloaking chamber under low pH conditions, and to prevent non-specific binding, tissues were blocked with 2.5% normal horse serum at room temperature for 5 minutes (R.T.U. Vectastain Kit; Vector Laboratories, Burlingame, CA). Incubation with a primary IgG2b mouse monoclonal antibody to human K17 (anti-K17 clone E3, obtained from both Abcam, Cambridge, MA and Invitrogen, Rockford, IL) was performed at 4 °C overnight. Negative controls included substitution of the primary antibody with non-immune mouse monoclonal IgG2b (Abcam, Cambridge, MA). The sections were then incubated with biotinylated horse anti-mouse/anti-rabbit IgG secondary antibody and R.T.U. Elite® ABC Reagent (R.T.U. Vectastain Kit; Vector Laboratories, Burlingame, CA) and staining was visualized using 3,3′-diaminobenzidine (Dako North America, Carpinteria, CA). K17 staining was scored based on the PathSQ Score, representing the percentage of tumor cells with strong K17 staining [8-11]. A single board-certified pathologist scored the cases and was blinded to the case clinical data. The PathSQ Score reflected only the epithelial components of carcinosarcomas since the sarcomatous components of these tumors were consistently negative for K17.

2.4. Statistical analysis

Patients were grouped into clinically meaningful categories for statistical analysis. For statistical comparisons of K17 with clinical variables, tumor stages were grouped as local (I + II) versus metastatic disease (III + IV). Censored events were those where a patient's last known status was “alive”,

regardless of cancer progression or recurrence, and failure events were defined as patient death. Continuous data were presented as mean ± standard deviation. Statistical comparison between two groups were performed using unpaired Student's *t* test or Mann-Whitney *U* test. Categorical data were analyzed using either Fisher exact test or χ^2 test.

The cut-off value for low versus high K17 mRNA and PathSQ score was based on the minimum Akaike's Information Criterion (AIC) from a Cox proportional-hazard regression model. The same cut-off values for mRNA and PathSQ Scores were utilized for all subsequent stratified analysis.

Survival analyses were completed using the Kaplan-Meier method. Univariate analyses were performed to compare patient survival within K17 status (high versus low), patient age at diagnosis, tumor stage (metastatic versus local), and histopathologic types (serous versus endometrioid, carcinosarcoma versus endometrioid). Univariate analysis, comparing patient survival between low and high K17 groups were also done while stratifying for tumor stage, and histopathologic types. Multivariate analyses using Cox proportional hazards regression were performed to examine overall survival while adjusting for potential confounders. All statistical testing was completed using SAS 9.4 (SAS Institute, Cary, NC) and GraphPad Prism (GraphPad Software, La Jolla, CA) with a $P = .05$.

3. Results

3.1. High K17 mRNA expression is associated with decreased overall survival

TCGA K17 mRNA expression values in high-grade endometrial carcinomas ranged from 3.65 to 190 218 TPM (transcripts per kilobase million) (Fig. 1A). The cut-off mRNA value to maximize the overall survival differences between the high versus low K17 groups was 1862 TPM, where tumors with >1862 TPM were classified as high K17 and those with ≤1862 TPM were grouped as low K17. Based on this threshold, the low K17 mRNA group comprised 61% (164/271) of cases, whereas high K17 mRNA group comprised 39% (107/271) of cases. Of the total 271 cases, 112 were grade 3 endometrioid carcinomas, 53 were carcinosarcomas, 93 were serous carcinomas, and 15 were of undefined high grade histological subtype. For high grade endometrioid carcinomas, 73% (82/112) were classified as low K17 and 27% (30/112) were classified as high K17. 49% (25/51) of carcinosarcomas were low K17 while 51% (26/51) were high K17. 49% (46/93) of serous carcinomas were low K17 and 51% (47/93) were high K17. Lastly, 73% (11/15) of the cases of undefined high grade histological subtype were low K17 and 27% (4/15) were high K17.

High K17 mRNA expression was associated with decreased overall survival among all high-grade endometrial cancer cases (HR = 1.802, $P = .0101$, Fig. 1B). Patients with high K17 mRNA had a median survival of 4.2 years while the median survival for patients with low K17 mRNA was 9.2 years (Fig. 1B). Univariate analysis revealed that overall survival was significantly different between tumors with different stage and histopathologic type (Fig. 1C, Supplementary Table 1). When adjusted for potential confounding factors using multivariate analysis, patient age and K17 status was no longer a significant predictor of survival, while cancer stage (HR = 4.379, $P < .0001$) remained significant (Fig. 1D). To determine survival differences between cases with the same tumor stage or histological subtype, we used stratified analysis by K17 mRNA status. There were no associations in K17 mRNA within either low or high stage tumors, or within each histological subtype. These results suggest that K17 may be negatively prognostic in high-grade endometrial cancers and warrants further investigation.

3.2. K17 mRNA expression does not correlate with TCGA molecular subgroups

The TCGA previously identified four molecular subgroups of endometrial cancer based on whole tissue sequencing: POLE (DNA polymerase epsilon, catalytic subunit mutation), MSI (microsatellite instability) status, copy-number low, and copy-number high, where patients with tumors in the copy-number high group having the poorest prognosis and patients with tumors in the POLE group having the best prognosis [19]. We did not find any association between K17 mRNA status (high versus low) and TCGA molecular subgroups (χ^2 test, $P = .3498$) nor between K17 mRNA levels and these subgroups (ANOVA, $P = .3126$) (Supplementary Fig. 1). Within the MSI group, K17 mRNA status was not prognostic for survival outcomes ($P = .9630$), nor was it prognostic in the copy-number high group ($P = .1387$) (Supplementary Table 2). There was not sufficient number of cases in the POLE and copy-number low groups to conclude any trends.

3.3. K17 IHC is detected in high-grade endometrial carcinoma

While the TCGA dataset provided K17 expression values from bulk-tumors lysates, IHC staining of tissue specimens was performed to better define K17 expression patterns at the cellular/histologic level. Although K17 was detected in malignant glandular cells of the endometrium (Fig. 2B-D), sections of endometrial stroma, myometrium, leiomyoma, leiomyosarcoma, or endometrial stroma sarcoma were uniformly negative for K17 (Fig. 2E-G). While the majority of benign endometrial glands were negative for K17, a few cases had minimal K17 staining (Fig. 2A, H). Within malignant glandular cells, K17 was detected predominantly in the cytoplasm of tumor cells, although punctate staining was also detected in the nucleus of some tumor cells, consistent with previously established findings that nuclear K17 impacts cell cycle progression in cancer (Fig. 3) [14,20].

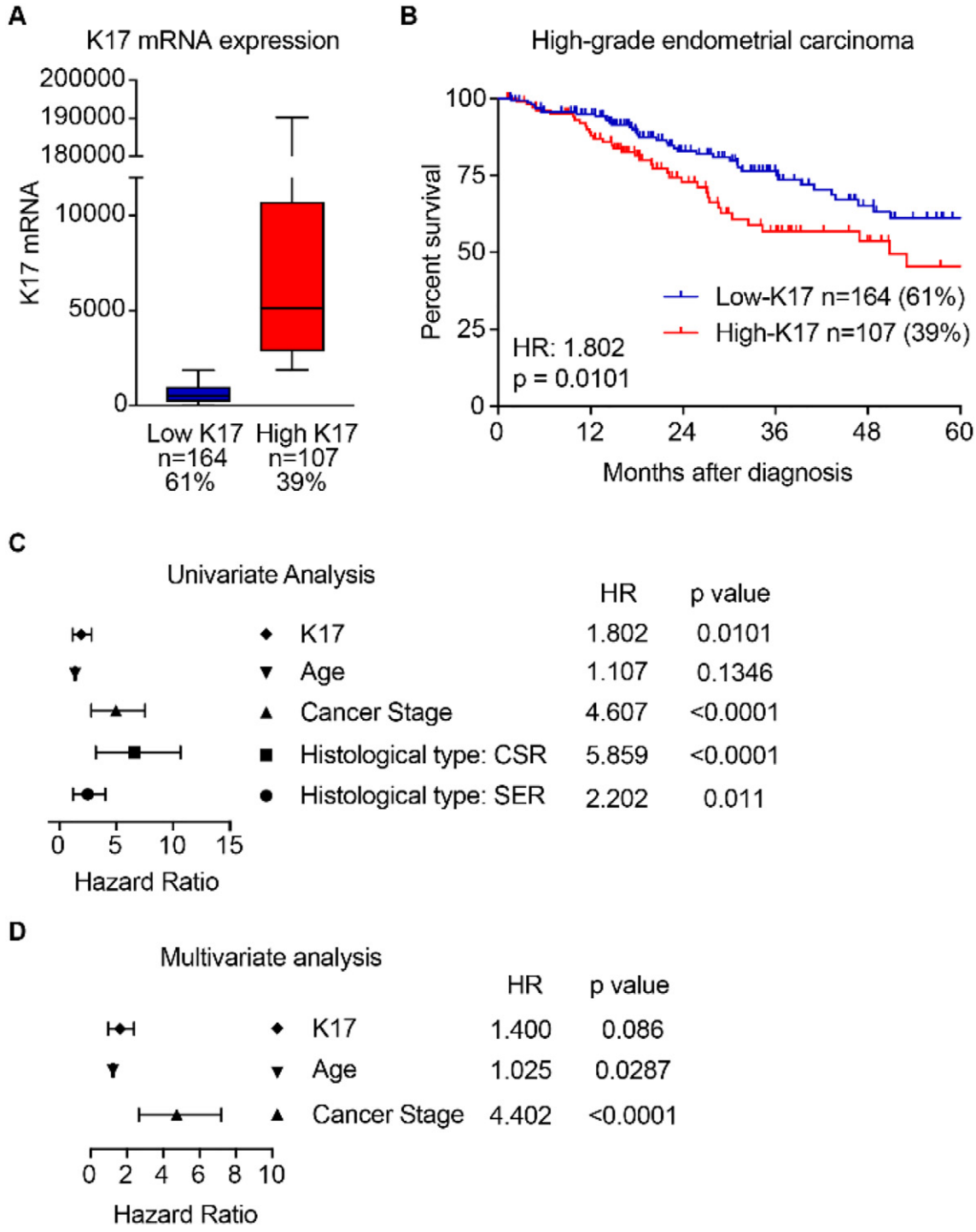

Fig. 1 High keratin 17 mRNA expression is associated with poor survival in patients with high-grade endometrial carcinoma. A, Distribution of keratin 17 (K17) mRNA expression in Low and High K17 groups. Gene expression quantified using RNA-Seq ranged from 3.65 to 190 218 TPM (transcripts per kilobase million) with an average of 4430 TPM and a standard deviation of 13 184 TPM. **B,** Kaplan–Meier curves for patients with high-grade endometrial cancer computed based on K17 mRNA status. **C,** Forest plots of univariate analysis for survival. Along with K17, stage (localized versus metastatic), and histological type (carcinosarcoma versus endometrioid, serous versus endometrioid) also correlated with poor survival outcomes. **D,** Multivariate analysis for survival. Stage (localized versus metastatic) and patient age were both independently prognostic; K17 mRNA was not prognostic. Hazard ratios are given with 95% confidence intervals. Abbreviations: HR, hazard ratio, CSR, carcinosarcoma, SER, serous carcinoma.

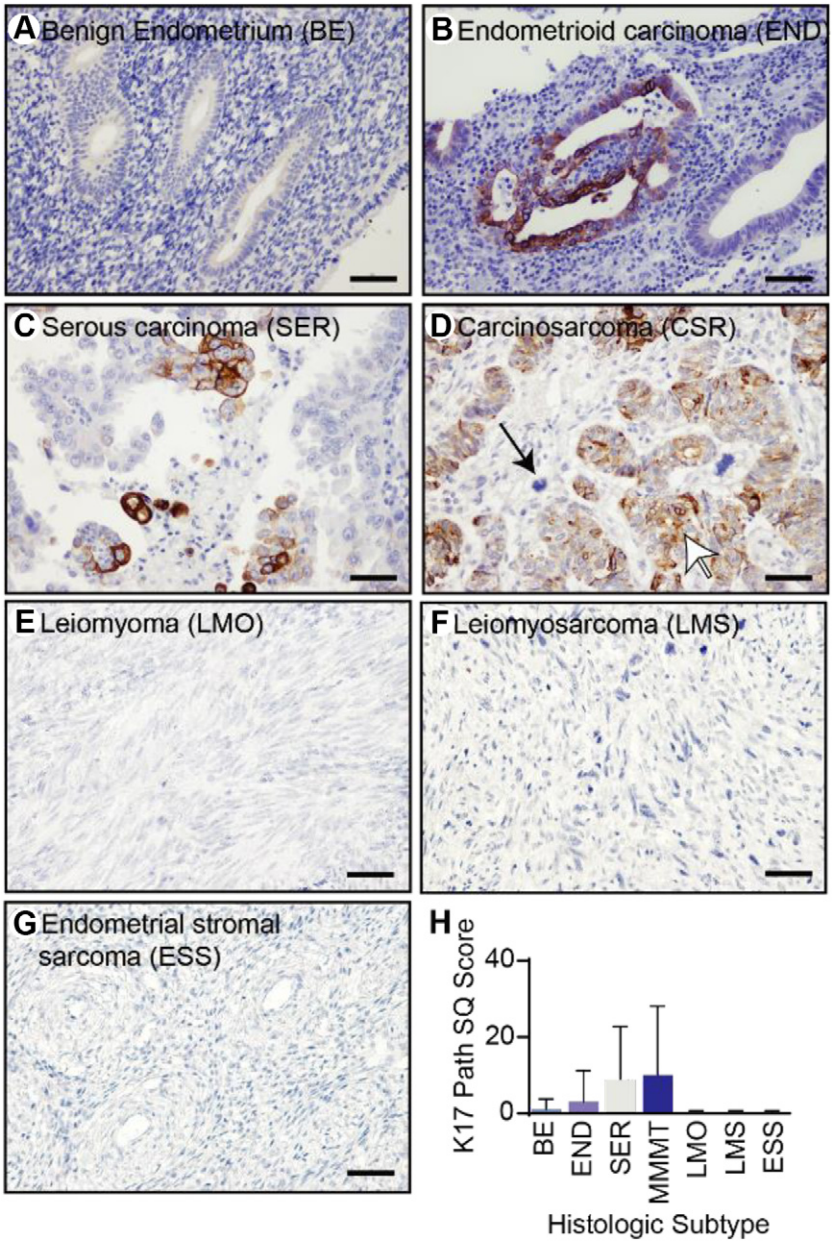

Fig. 2 Keratin 17 is detected predominantly in malignant epithelial tumors of the endometrium. A, Benign endometrium; **B,** Endometrioid carcinoma; **C,** Serous carcinoma; **D,** Carcinosarcoma; white arrow depicts carcinomatous component, black arrow depicts mesenchymal component; **E,** Leiomyoma; **F,** Leiomyosarcoma; and **G,** Endometrial stromal sarcoma. H, Average K17 PathSQ score by histological subtype, error bars represent 1 SD. All images are at 400×, with scale bars represent 50 μm. Abbreviations: BE, benign endometrium, END, endometrioid carcinoma, SER, serous carcinoma, CSR, carcinosarcoma, LMO, leiomyoma, LMS, leiomyosarcoma, ESS, endometrial stromal sarcoma, SD, standard deviation.

Overall, K17 shows a heterogeneous staining pattern in tissue. Malignant glandular cells were either strongly stained for K17 or not stained at all. In cases with a large number of K17 positive cells, there was intense staining in the malignant glandular cells in the periphery of the tumor at the tumor-stromal interphase and in those that showed squamous differentiation; K17 staining can be detected in up to 95% of tumor cells in these areas (Supplementary Fig. 2A). There was also high-level staining noted in single tumor cells and small groups of cells that diffusely infiltrating the myometrium (Supplementary Fig. 2B). Malignant glandular cells in the central areas of the tumor and those that line the uterine cavity, on the other hand, are less likely to have K17 staining detected, regardless of the histological type or the overall K17 status of the case. Additionally, while the epithelial components of carcinosarcomas often showed high levels of K17 expression, there was minimal staining in the

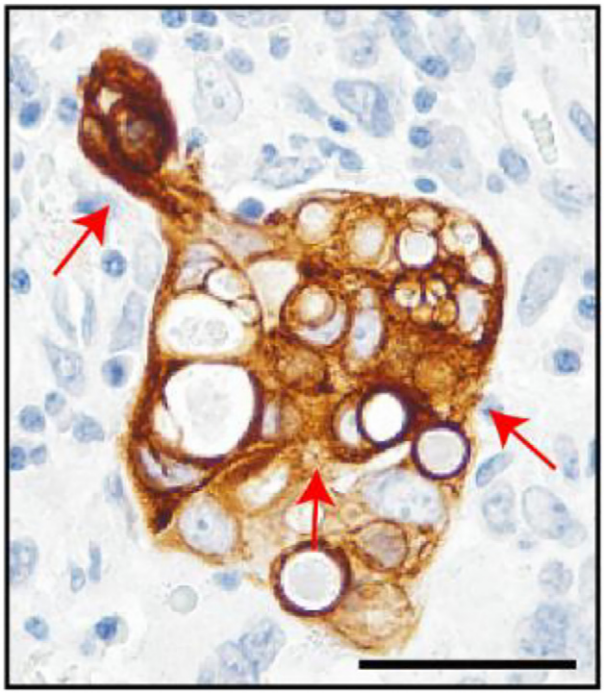

Fig. 3 Keratin 17 is mainly expressed in the cytoplasm of malignant endometrial glands, although nuclear staining is also detected in some cells. Representative image showing cytoplasmic and nuclear keratin 17 staining (red arrows) in the epithelial component of a carcinosarcoma. Magnification 400×, scale bar represents 50 μm.

sarcomatous components of these tumors. These findings indicate that K17 IHC highlights malignant epithelial cells of the endometrium, consistent with previous reports that K17 expression is detected in malignant epithelial cells and is associated with tumor aggression [8,9,12,14,21].

The portion of K17 positive tumor cells for the IHC cohort ranged from 0% to 95% (PathSQ Score) (Fig. 4A-C). The cut-off PathSQ Scores for high and low K17 were ≥10% and <10%, respectively. This cut-off score was determined in a similar method to that of the mRNA cut-off value, in order to maximize the survival differences between the two groups. Based on this threshold, low K17 comprised of 71% (84/119) of cases, including 100% (5/5) of grade 3 endometrioid carcinomas, 71% (44/62) of serous carcinomas, and 67% (35/52) of carcinosarcomas. Similarly, high K17 comprised 29% (35/119) of cases, including 0/5 grade 3 endometrioid carcinomas, 29% (18/62) of serous carcinoma, and 33% (17/52) of carcinosarcomas.

3.4. K17 IHC was prognostic for decreased survival in high-grade endometrial carcinomas

High K17 IHC expression was associated with decreased survival in patients with endometrial cancer, with a median survival of 1.7 years (HR = 1.752, P = .0488, Fig. 4D). Univariate analyses showed that overall survival was significantly different between tumor stage and histopathologic type, but not patient age at diagnosis (Fig. 4E, Supplementary Table 1). Additionally, the overall median survival time was shorter in the high K17 cases (1.7 years) compared with the low K17 group (4.6 years). When adjusted for potential confounding factors using multivariate analysis, K17 IHC staining was a significant and independent predictor of survival (HR = 1.996, P = .0192), along with cancer stage (HR = 2.565, P = .0009, Fig. 4F). These results demonstrate that K17 IHC is a negative prognostic marker in high-grade endometrial carcinomas and is independent of tumor stage.

3.5. K17 IHC outperformed mRNA as an independent prognostic biomarker for high-grade endometrial cancers

The hazard ratios (HR) for K17 IHC were higher than the HR for K17 mRNA for the multivariate analyses (mRNA univariate, HR: 1.802, P = .0101; multivariate, HR: 1.400, P = .0860; IHC univariate, HR: 1.752, P = .0488; multivariate, HR: 1.996, P = .0192) (Figs. 1C-D, 4E-F). Multivariate analysis also revealed that K17 IHC was independently prognostic, while K17 mRNA was not. In a sub-analysis of patients with early stage cancers, neither K17 mRNA nor K17 IHC was predictive of survival outcomes (mRNA, HR: 1.682, P = .1923; IHC, HR: 2.053, P = .1052, Table 2), and this continued to be the case for patients with metastatic disease (mRNA, HR: 1.479, P = .1964; IHC, HR: 1.750, P = .1155, Table 2). Thus, compared to K17 mRNA, K17 IHC provides additional prognostic value and more robust survival curve separation in high-grade endometrial cancers.

3.6. Benign endometrial processes, low-grade endometrioid carcinomas, clear-cell carcinomas, and uterine sarcomas showed minimal K17 IHC staining

Benign endometrial conditions such as leiomyoma (n = 9), atrophic endometrium, (n = 18) secretory (n = 12) and proliferative endometrium (n = 11) all showed minimal K17 staining, along with maligiant endometrial processes such as endometrial stromal sarcomas (n = 9) and leiomyosarcomas (n = 13) (Fig. 2A, E-H).

The type 1 endometrial carcinomas (n = 82) revealed minimal K17 IHC staining as well. Only 4% (3/82) of the cases (grade 1, 2/46; grade 2, 1/36) were revealed to have high K17. The last known status at follow up for all three of these patients were alive. Thus, we cannot assess whether or not K17 IHC predicts survival outcomes in this histological subtype of endometrial cancer. Similarly, only 15% (6/41) of clear cell carcinomas were high K17; further studies need to be performed to better evaluate whether K17 status is prognostic in this subtype of endometrial cancer.

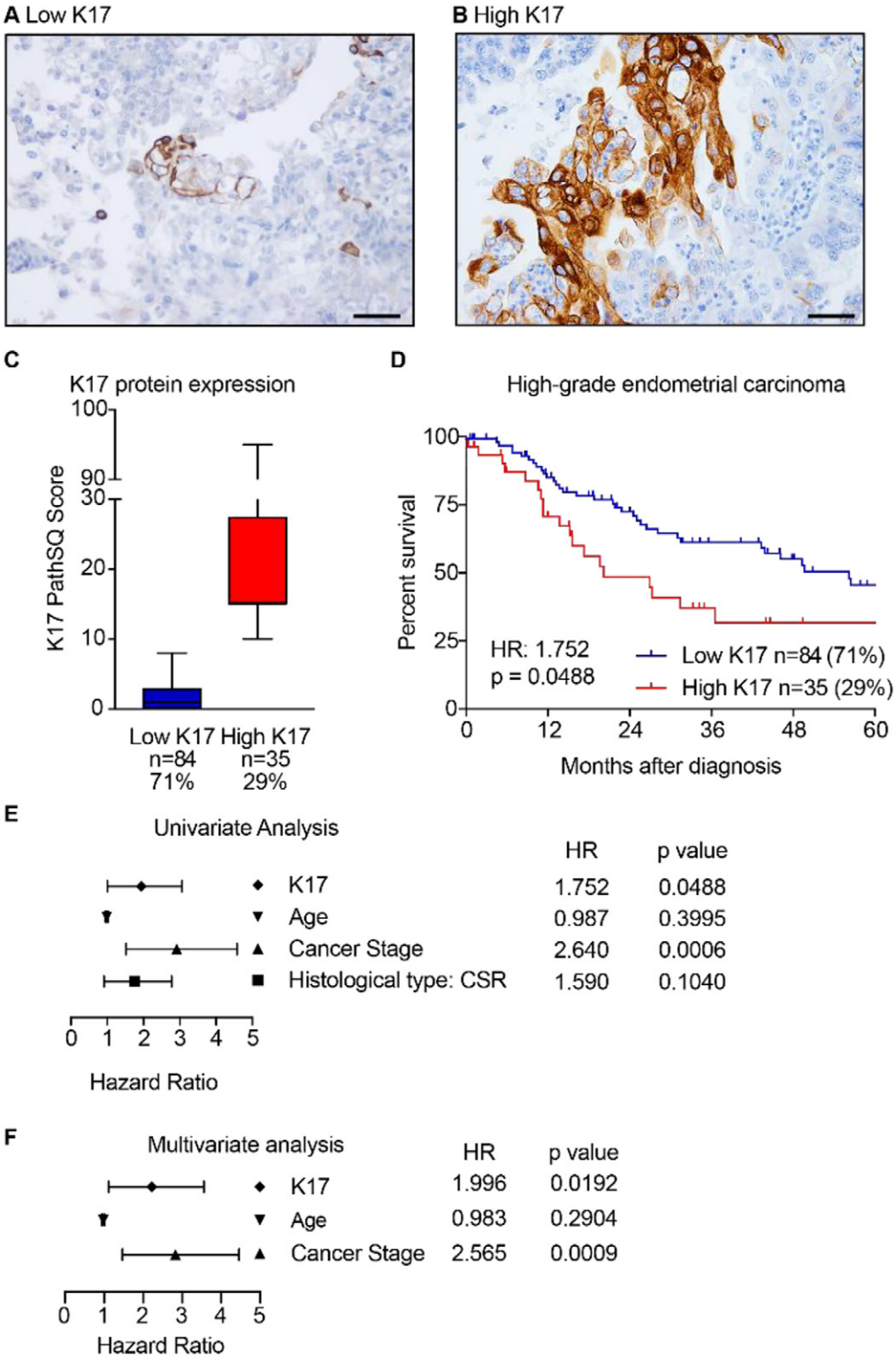

Fig. 4 Immunohistochemistry (IHC) for K17 is an independent predictor of poor survival in patients with high-grade endometrial carcinoma. A and B, Representative images of keratin 17 (K17) IHC. **C,** The PathSQ IHC Score ranged from 0% to 95% overall, with an average of 9.2% and standard deviation of 15.6%. **D,** Kaplan–Meier curves for patients with endometrial cancer computed using K17 protein status. **E,** Forest plots of univariate analysis for survival. Along with K17, cancer stage (localized versus metastatic) also correlated with poor survival outcomes. Patient age and histological type (carcinosarcoma versus serous) did not correlate with survival outcomes. **F,** Multivariate analysis for survival. K17 and stage (localized versus metastatic) were independently prognostic. Hazard ratios are given with 95% confidence intervals. Abbreviations: HR, hazard ratio; CSR, carcinosarcoma, serous, serous carcinoma. Both histologic images are at 400×, scale bars represent 50 μm.

Table 2 Keratin 17 (K17) immunohistochemistry (IHC) is associated with poor survival outcomes in high-grade endometrial carcinomas

Group	Cohort 1: K17 mRNA [a]				Cohort 2: K17 IHC [b]			
	High K17/ Total N (%)	HR [c]	95% CI for HR	*P*	High K17/ Total N (%)	HR [c]	95% CI for HR	*P*
Clinical stage [d]								
I/II	54/160 (34)	1.682	0.7050–4.012	.1923	14/63 (22)	2.053	0.7001–6.018	.1052
III/IV	53/110 (48)	1.479	0.8555–2.557	.1964	20/51 (39)	1.750	0.7784–3.886	.1155
N/A [e]	0/1 (0)	N/A	N/A	N/A	1/5 (20)	N/A	N/A	N/A
Histological type								
High-grade	107/271 (39)	1.802	1.150–2.822	.0101	35/119 (29)	1.752	1.003–3.061	.0488
Endometrioid	30/112 (27)	1.846	0.5945–5.730	.2194	0/5 (0)	N/A	N/A	N/A
Serous	47/93 (51)	1.810	0.8472–3.865	.1155	18/62 (29)	1.791	0.6359–5.044	.1997
Carcinosarcoma	26/51 (51)	0.9180	0.4485–1.879	.8144	17/52 (33)	1.671	0.7841–3.563	.1199

Abbreviations: CI, confidence interval; IHC, immunohistochemistry; N/A, not available.

[a] Gene expression data were obtained from The Cancer Genome Atlas (TCGA).

[b] Endometrial cancer tissues were obtained from the Stony Brook (SB) Medicine Cancer Registry and the UMass Memorial Cancer Registry.

[c] Log-rank Hazard Ratio and CI.

[d] Stage I/II: local disease; III/IV: metastatic disease.

[e] Clinical stage was unknown for 1 case from the TCGA database. Clinical stage was unknown for 5 cases from the Stony Brook Medicine Cancer Registry; patients had hysterectomy prior to 1998 and original pathology reports were not available.

4. Discussion

In this study, we demonstrate that K17 is a significant independent predictor of survival in patients with high-grade endometrial carcinoma, and to our knowledge, this is the first study to evaluate K17 as a prognostic biomarker in uterine cancer.

K17 is principally expressed in the cytoplasm of malignant glandular cells of the endometrium, but not in benign stroma or glandular cells. Proliferating smooth muscle cells, benign or malignant, do not express K17, as no staining were detected in leiomyoma, leiomyosarcoma, and endometrial stromal sarcoma. Cancer stage is also an independent prognostic factor in this study, as expected. The groups with the highest proportion of high K17 cases were papillary serous carcinomas and carcinosarcomas and in this combined group, high K17 was a negative prognostic biomarker. This result did not reach statistical significance when these cases were separated into separate diagnostic categories, potentially indicating that the study was underpowered to define this association. We found no evidence to suggest that K17 is prognostic in endometrioid or clear cell carcinomas, in part because the K17 was detected at only low levels in these cases. We were also unable to assess whether or not K17 IHC is prognostic in poorly differentiated (ie, grade 3) endometrioid carcinomas, due to a lack of cases. Thus, we found that K17 is associated with decreased survival in high-grade endometrial carcinomas, as has been reported in various other carcinomas [7-11].

In contrast to epithelial ovarian cancer and breast cancer, where K17 mRNA expression is highly correlated with IHC staining, this study found some notable discrepancies between K17 mRNA and protein results [7,9]. For example, the median survival time was 4.2 years for patients with high K17 mRNA tumors in the TCGA cohort compared to 1.7 years for the high K17 in the IHC cohort. Furthermore, K17 IHC was not only an independent prognostic marker, but was also nearly as strong a predictor for survival as stage, whereas K17 mRNA was not prognostic (Figs. 1D, 4F). High K17 endometrial cancers accounted for 39% of the cases in TCGA, whereas only 29% of cases showed high expression of K17 in our IHC series. These differences in the proportion of high K17 groups may be attributed to differences in case representation between the two datasets, rather than differences in the established mRNA or PathSQ cut-off values, as they were both determined using the same AIC method. For example, endometrioid carcinomas comprise of 41% of the TCGA cohort, compared to 3% of the IHC cohort. The TCGA has an abundance of poorly differentiated endometrioid carcinomas (n = 112); by contrast, there was a dearth of poorly differentiated endometrioid carcinomas in the IHC cohort (n = 5). Together, these differences in the TCGA and IHC study cohorts could explain the differences we observed in the proportion of low and high K17 groups between the two cohorts.

Recent development of tumor sequencing projects lead to the exploration, identification, and subsequent confirmation of molecular subtypes of endometrial cancer based on mutations and copy number variations [19,22,23]. Levine proposed four molecular subgroups of endometrial carcinoma: POLE (mutations in DNA polymerase epsilon, catalytic subunit), MSI (microsatellite instability) status, copy-number low, and copy-number high, where the POLE group had the best prognosis and copy-number high had the worst [19]. Upon comparison of K17 mRNA status and levels between each of these molecular groups, we failed to find a significant association. We believe this disconnect could be due to the fact that endometrial carcinomas are histologically diverse and the proportion of tumor cells within a given volume of tumor may show high levels of variability within and across different cases. Thus, the

processing of tissues as whole tissue lysates, as used for RNAseq studies in TCGA database, is impacted by dilution of tumor cell-derived mRNA and mRNA derived from other non-neoplastic cellular elements, potentially including benign stromal cells, smooth muscle, endothelial cells, and inflammatory cells. By contrast, IHC enables a more precise analysis of biomarker expression by the malignant cellular components of a given tumor that is not impacted by the presence of other cellular tissue components. Additionally, endometrioid carcinoma, the most common histologic type of uterine cancer accounting for 75% to 80% of all endometrial cancer, is represented by 41% of the cases in TCGA [4]. The IHC cohort was constructed to provide a roughly equivalent number of high-grade cases within each major histopathologic type. The differences in the approach of quantifying K17 via mRNA versus IHC offer an explanation regarding the differences in the findings between the TCGA and IHC cohorts. Furthermore, these differences also highlight both the caveats of large-scale genome sequencing and the importance of protein validation studies when evaluating protein biomarkers.

While keratins have historically been thought to function primarily as structural proteins that provide mechanical support, it is now established that K17, uniquely among keratins, has diverse roles in regulation of processes that are central to the pathogenesis of both inflammation and malignancy [7,8,14,20,24-26]. Normally expressed in hair follicles, nails, and sweat glands, K17 drives the pathogenesis of psoriasis by stimulating the production of cytokines in autoreactive T-cells and regulates epithelial cell growth, in response to tissue injury, by binding and translocating nuclear 14-3-3σ into the cytoplasm [24,27]. In cancer, one mechanism by which K17 drives oncogenic transformation and uncontrolled cell proliferation is via nuclear localization and export of the cell cycle inhibitor, p27 [14,20]. This process may also occur in endometrial carcinoma, as studies have described a reduction of p27 expression in both serous carcinoma and carcinosarcoma, compared with benign endometrial tissue [28,29]. K17 is also overexpressed in several other epithelial and soft-tissue malignancies, including pancreatic adenocarcinoma, pulmonary adenocarcinoma, basal cell carcinoma, bi-phasic synovial sarcoma, and Ewing sarcoma, suggesting that K17 may drive tumorigenesis via multiple pathways [8,9,25,30-32]. Furthermore, K17 induces inflammatory cytokine production leading to epithelial hyperplasia in basal cell carcinoma and regulates cellular adhesion via activation of AKT/PKB in Ewing sarcoma [25,32]. Future studies should aim to further explore how K17 impacts tumor aggression in high-grade endometrial carcinomas, either via regulation of cell cycle regulators such as p27 or through activation of inflammatory pathways.

One limitation of our current study is that our quantifications of K17 gene expression and immunohistochemistry profiling did not originate from the same cohort, thus we cannot directly compare the results from these two cohorts nor can we make definitive conclusions of the relationship between K17 mRNA and protein expression. The fact that K17 IHC status was significant for survival for the entire cohort but not for the individual subtypes suggests that our study could have been underpowered to detect a difference in survival for each histological subtype of endometrial cancer. Additionally, our immunohistochemical scoring systems do not account for the variability in biomarker expression across cases. For example, we observed some cases where the great majority of tumor cells did not express K17 but when we focused on only the diffusely infiltrative population of tumor cells, almost all were strongly positive for K17 (Supplementary Fig. 2B). A scoring system that does not take into account the histologic variability in staining patterns could underestimate the potential effects of biomarker expression within discrete populations of tumor cells. The "event" we used in our analysis is overall survival, which could be confounded by patient deaths unrelated to cancer. It is important to note, however, that reanalysis of the data revelated that K17 IHC staining also correlates with progression-free survival in a similar manner (data not shown). Lastly, this study did not control for ethnicity, family history of gynecologic malignancies, history of hormone use (either for contraception or menopause), history of estrogen receptor modulator use, and administration of adjuvant treatment.

In summary, this study provides evidence that K17 IHC is an independent prognostic biomarker in patients with high-grade endometrial carcinoma. In carcinosarcoma cases, scoring of K17 status should be determined solely based on staining of the carcinomatous component. Thus, K17 IHC could be used to supplement well established prognostic indicators of endometrial cancer patient survival, including stage, to predict outcome. It remains almost entirely unexplored, however, whether K17 could also be exploited as a pharmacologic target to extend survival of endometrial cancer patients with high-grade, high-K17 carcinomas.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2019.09.005.

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