Published in final edited form as:

Am J Surg Pathol. 2010 July ; 34(7): 915–926. doi:10.1097/PAS.0b013e3181e3291e.

A Panel of 3 Markers Including p16, ProExC, or HPV ISH is Optimal for Distinguishing Between Primary Endometrial and Endocervical Adenocarcinomas

Christina S. Kong, MD, Andrew H. Beck, MD, and Teri A. Longacre, MD

Department of Pathology, Stanford University School of Medicine, Stanford, CA

Abstract

Endometrial and endocervical adenocarcinomas may seem histologically identical and it can be difficult to determine primary site of origin based on morphology alone. As the distinction is significant and cannot always be made on the basis of clinical findings, various immunohistochemical panels have been proposed to aid in determining site of origin. Stains for vimentin, estrogen receptor (ER), progesterone receptor (PR), monoclonal carcinoembryonic antigen, p16 and ProExC, and HPV in situ hybridization (ISH), were performed on 283 tissue microarray (TMA) cores and 38 whole sections. The TMA consisted of 214 endometrial carcinomas, 33 endocervical adenocarcinomas, and 36 problematic cases. The endometrial and endocervical carcinomas represented usual endometrioid and mucinous types, and special variants (uterine serous carcinoma, uterine clear cell carcinoma, minimal deviation endocervical adenocarcinoma, cervical small cell carcinoma, adenoid basal cell carcinoma, mesonephric carcinoma). Univariate analysis showed that 6 markers (vimentin, ER, PR, p16, ProExC, and HPV ISH) performed well in distinguishing between endocervical and endometrial origin for the usual endometrioid and mucinous types. Multivariate analysis showed that vimentin, p16, and HPV ISH are the strongest predictors of site. Using a script written in R, the diagnostic accuracy of all possible combinations of markers was evaluated and it was shown that a 3 marker panel including vimentin, ER, or PR, and an HPV marker (p16, ProExC, or HPV ISH) is optimal for determining site of origin for usual endometrial and endocervical adenocarcinomas. However, these panels do not perform well with special variant carcinomas.

Keywords

adenocarcinoma; endocervix; endometrium; p16; ProExC; HPV ISH; estrogen receptor; progesterone receptor; monoclonal CEA; vimentin; minimal deviation; adenoma malignum

Endocervical and endometrial adenocarcinomas have significant morphologic overlap and in cases in which a background of endocervical adenocarcinoma in situ or endometrial hyperplasia is not identified, it can be especially difficult to distinguish between the 2 sites. The distinction is clinically significant and important to make preoperatively as surgical

Correspondence: Christina S. Kong, MD, Department of Pathology, Stanford University School of Medicine 300 Pasteur Drive, Room L235, Stanford, CA 94305-5324 (ckong@stanford.edu).

This work was presented in part at the USCAP meeting in San Antonio March 2005.

management and postoperative decisions about chemotherapy and radiation therapy are driven by site of origin. Studies that can be helpful in making this distinction include physical examination, fractional curettage, imaging studies, histologic features, and immunohistochemistry. Histologic features that favor endocervical origin include eosinophilic fibrotic stroma, apical mitotic figures, basal apoptotic bodies, presence of adenocarcinoma in situ or squamous dysplasia, and monomorphous appearance. Features that favor endometrial origin include the presence of endometrial stromal or foam cells, complex endometrial hyperplasia, and polymorphous appearance. Although highly specific, foam cells are not frequently encountered in endometrial carcinomas. In addition, while the merging of carcinoma with typical endocervical or endometrial epithelium may suggest origin from those sites, these findings may also represent colonization of normal tissue by tumor and are not reliable predictors of the site of origin. In some cases when biopsy or curettage specimens consist entirely of fragments of tumor, it is virtually impossible to determine the site of origin based on morphology alone. The problem is compounded by tumors that straddle the lower uterine segment and endocervix; in these cases, even with hysterectomy specimens, it can be difficult to identify where the tumor originated.

Several studies have evaluated the use of estrogen and progesterone receptors, vimentin, mCEA, p16, and HPV in situ hybridization (ISH) in distinguishing endocervical from endometrial origin (Table 1). The different recommended panels include ER, vimentin, CK8/18 and CEA²; vimentin, and ER¹⁸; HPV ISH or p16 and ER/PR^{29,33}; CEA, vimentin and ER²³; vimentin, and CEA.^{6,10} Before the identification of HPV as a probable etiologic agent in the development of endocervical adenocarcinomas and the advent of commercially available surrogate markers for detecting HPV, most of the immunohistochemical markers in these panels, such as estrogen and progesterone receptors and vimentin, targeted endometrial adenocarcinomas. Carcinoembryonic antigen (CEA) was the only positive marker for endocervical adenocarcinomas. However, the use of CEA is significantly limited by the high degree of variability in results depending on the methodology and antibody used.²⁹ Moreover, the development of these panels focused primarily on straightforward cases of endocervical and endometrial carcinomas and evaluated a limited set of markers. Few studies have tested the use of a panel derived from an extensive set of markers on a separate set of more diagnostically difficult cases.^{29,34}

More recent studies have exploited the role of HPV in endocervical adenocarcinomas by using HPV ISH or the surrogate marker p16 to identify the presence of high-risk HPV. These studies have also suggested that p16 may be a useful addition to the panel for distinguishing endocervical from endometrial primaries (Table 1).^{3,22,27,29,33,34} Similar to p16, ProExC has been proposed as an additional surrogate marker for high-risk HPV but it is not nearly as well-established or studied as p16. ProExC targets cell cycle proteins, MCM2, and TOP2A, which are overexpressed when viral DNA integrates into the host genome leading to increased levels of E6 and E7 and aberrant S-phase induction.^{5,8,9,19,28} Several studies have evaluated the performance of ProExC in detecting HPV-related cervical squamous intraepithelial lesions^{5,8,9,25,31} and one has examined the differential expression of ProExC in endocervical adenocarcinomas and benign glandular mimics.⁴ However, no studies to date have examined the use of ProExC in distinguishing endocervical from endometrial adenocarcinomas.

In this study, we evaluate the performance of a series of markers for HPV (p16, ProExC, HPV ISH) and traditional immunohistochemical markers (ER, PR, vimentin, mCEA) in distinguishing endocervical from endometrial site of origin in tissue microarrays and whole sections. In addition to evaluating the usual types of endometrioid and mucinous adenocarcinomas, we included special variants such as minimal deviation endocervical adenocarcinoma, uterine serous carcinoma, and uterine clear cell carcinoma, as well as a set of problematic cases.

MATERIALS AND METHODS

Case Set Selection

The surgical pathology archives at Stanford Hospital and Clinics were searched from October 1, 1995 to June 30, 2005 for patients with a diagnosis of endometrial carcinoma, endocervical adenocarcinoma, and endocervical adenocarcinoma in situ. A separate search was performed for minimal deviation carcinoma of the endocervix. Specimen types included endocervical curettage/ biopsy, cervical cone biopsy, cervical LEEP, endometrial curettage/ biopsy, and hysterectomy. A total of 302 cases were identified: 217 endometrial carcinomas, 44 endocervical adenocarcinomas, and 41 problem cases. The problem cases lacked a component of endocervical adenocarcinoma in situ or background of endometrial hyperplasia, or exhibited carcinoma in both endocervical curettage and endometrial biopsy specimens, or straddled the lower uterine segment in hysterectomy specimens. Determination of site of origin for the problem cases was based on subsequent hysterectomy specimens, when available. In cases in which the tumor straddled the lower uterine segment and endocervix, the primary site was assigned based on the location of the tumor bulk and correlation with clinical and radiographic findings. In cases in which hysterectomy was not performed, determination was based on clinical and radiographic findings.

Tissue Microarray

The tissue microarray was constructed by using a tissue arrayer (Beecher Instruments, Silver Spring, MD) to create 2 new paraffin blocks from representative 0.6-mm cores taken in triplicate from 285 paraffin blocks of endocervical adenocarcinomas, endometrial carcinomas, and problem cases.²¹ The samples used to construct the tissue microarray consisted of 214 endometrial carcinomas, 33 endocervical adenocarcinomas, and 36 problem cases. The endometrial carcinomas were predominantly of the endometrioid and mucinous types (197/214, pure or mixed) and ranged from well-differentiated to poorly differentiated; 17/214 endometrial cases were special variants (11 serous, 6 clear cell). The endocervical carcinomas consisted of 20 cases of invasive adenocarcinoma (endometrioid and mucinous types) and 13 cases of adenocarcinoma in situ. The microarrays also included control cores of placenta.

Routine Tissue Sections

Routine whole tissue sections of a subset of cases (38/302) were evaluated. Of 38 cases, 19 (4 endometrial, 6 endocervical, 9 problem) were also present in the tissue microarray. The remaining 19 cases were unique and consisted of 3 endometrial carcinomas (1 endometrioid, 2 serous), 11 special variant endocervical carcinomas (8 minimal deviation, 1 small cell, 1

adenoid basal cell, 1 mesonephric), and 5 problem cases. The minimal deviation endocervical adenocarcinomas cases consisted of mucinous, endometrioid, and/or nonspecific cell types.²⁰

In addition, whole sections from 4/34 classic endocervical adenocarcinomas and 8/16 problem endocervical cases (site of origin determined based on gross, clinical, and/or radiographic findings) were stained with p16. These cases were present in the tissue microarray and were further evaluated due to equivocal p16 results. p16 stain was performed twice on tissue microarray sections to confirm the equivocal results. ProExC was also equivocal in a similar subset of cases but stains on whole sections were not performed.

Immunohistochemistry

Immunohistochemical analysis was done with these commercially available antibodies: estrogen receptor (ER), progesterone receptor (PR), monoclonal carcinoembryonic antigen (mCEA), vimentin, p16, and ProExC (Table 2). Four μ M thick sections were cut from the tissue microarray blocks and routine paraffin-embedded blocks of tumor then deparaffinized in xylene, and hydrated in graded alcohols. ER, PR, vimentin, p16, and ProExC stains were performed with the Dako Autostainer (Glostrup, Denmark) whereas mCEA was done with no antigen retrieval on the Benchmark Automated Slide Stainer (Tucson, AZ). For stains performed on the Dako Autostainer, the slides were incubated with peroxidase-blocking reagent, followed by the primary antibody (dilution 1:50) then the visualization reagent (secondary goat-antimouse immunoglobulin and horseradish peroxidase linked to a dextran polymer backbone). After rinsing with distilled water, the slides were incubated with DAB (3, 3'-diaminobenzidine) substrate- chromagen solution and a Mayer hematoxylin counterstain was applied before coverslipping.

A paraffin-embedded section of a tissue microarray of normal and neoplastic tissue was used as an external positive control for ER, PR, mCEA, and vimentin. A paraffin-embedded section of HSIL confirmed to be positive for high-risk HPV by PCR was used as an external positive control for p16 and ProExC. In addition, negative control serum was applied to sections of the microarray and HSIL as negative controls.

For ER, PR, vimentin, and mCEA, the staining was scored as strong (3), weak (2), equivocal (1), or negative (0). Nuclear staining was scored as positive for ER and PR; membranous and/or cytoplasmic staining, for vimentin. For mCEA, luminal, and cytoplasmic staining were scored independently. For ProExC and p16, the staining was scored as diffuse (>80%) strong (3), focal (5% to 80%) strong (2), equivocal (1) or negative (0) based on nuclear staining for ProExC and nuclear or nuclear and cytoplasmic staining for p16. For ProExC and p16, weak cytoplasmic staining or reactivity in <5% nuclei was interpreted as negative.

HPV In Situ Hybridization

The INFORM HPV III and HPV III Family 16 ISH assays were done with the Benchmark Automated Slide Stainer, utilizing high-risk HPV probes that detect HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 68, and 70. The HPV III ISH probe is no longer commercially available and has been replaced by the HPV III Family16 probe (HPV 16). HPV III ISH was performed on 4 μ M-thick sections of the tissue microarray and a subset (16/ 38) of the

routine whole sections. HPV16 ISH was performed on whole sections of the special variant endocervical adenocarcinomas. Both ISH assays were done on 4 µm-thick sections with the Benchmark Automated Slide Stainer. The automated system uses the Ventana ISH Protease 3 (8 min) to remove proteins surrounding the target DNA, the above-mentioned high-risk HPV probe and the Ventana *VIEW*_{Blue} detection kit. The detection system uses a biotinylated anti fluorescein antibody to detect the hybridized probe followed by streptavidin to bind biotin then a chromagen reaction with NBP (nitroblue tetrazolium) and BCIP (5-bromo-4-chloro-3-indolyl-phosphate) for detection. Counterstain was done with Ventana Red Counterstain II (4 min), which is based on a nuclear fast red stain. After staining, the slides were put through a dehydration procedure before coverslipping. A paraffin-embedded section of HSIL confirmed to be positive for high-risk HPV by PCR was used as an external positive control. In addition, negative control serum was applied to a section of HSIL as a negative control. Punctate and/or diffuse nuclear staining was scored as negative (0), uninterpretable (1), weak (2) or strong (3).

Data Analysis

The data was analyzed by 2 different methods. Problem cases and special variants (eg, serous carcinoma, clear cell carcinoma, minimal deviation) were excluded and evaluated separately from the classic cases of endometrial and mucinous types of endocervical and endometrial carcinoma.

For the first evaluation, the tissue microarray nonproblem cases were divided into a training set (108/230) and test set (122/230) consisting of both endometrioid and mucinous types of endometrial and endocervical adenocarcinomas in each set. Univariate analysis was done on the training set with SPSS17.0 software to determine the performance of each marker in distinguishing between endocervical and endometrial origin. Using the 6 markers (p16, ProExC, HPV ISH, ER, PR, vimentin) that showed a significant association with primary tumor site on the training cases, we built a multivariate model using logistic regression on the training cases and evaluated the multivariate model's ability to predict tumor site on the test set of cases.

As the logistic regression analysis produced a complex statistical model (which may be difficult to apply in routine clinical practice), the second evaluation attempted to create a simpler model that weighted each contributing stain equally. Should a simpler model perform comparably to the logistic regression model, it would be preferable as it would be easier to apply in clinical practice. To perform this analysis, we computed the overall diagnostic accuracy, diagnostic accuracy for cervical primary site, and diagnostic accuracy for endometrial primary site for all combinations of the 6 markers that showed the best performance in the univariate analysis (p16, ProExC, HPV ISH, ER, PR, vimentin). For this analysis, all results were binarized so that only diffuse strong staining for p16 or ProExC was considered positive, whereas both weak and strong HPV ISH, vimentin, ER, and PR were scored as positive. Classic cases and problem cases were evaluated separately. A script was written in the R programming language¹⁷ to compute the diagnostic accuracy for all possible combinations of markers and all possible diagnostic cut-offs for this analysis (This

program can be generalized for use in other studies designed to identify the best performing panel of markers and diagnostic threshold, and is available upon request).

RESULTS

Univariate and Multivariate Analysis

This analysis includes all 230 TMA cases that were classic endometrial or endocervical. The cases were heavily weighted toward endometrial primary site, with approximately 85% of cases from endometrial site and approximately 15% of cases from cervical. This bias reflects the approximate distribution seen in clinical practice, in which the majority of adenocarcinomas identified in the uterus/ lower uterine segment are of endometrial origin with a minority originating from the endocervix.

Univariate analysis on the training set identified 6 markers—p16, ProExC, ER, PR, vimentin, and HPV ISH—as performing well in distinguishing between endocervical and endometrial origin. Positive results for vimentin, ER, and PR support endometrial origin whereas p16, ProExC, and HPV ISH support endocervical origin (Figs. 1, 2). For p16 and ProExC, diffuse strong staining (score 3) was required for good performance whereas HPV ISH performed best if both weak and strong staining were scored as positive. ER, PR, and vimentin performed well regardless of the cut-off used. Cytoplasmic and/or luminal mCEA was not useful, and mCEA staining was not included in the multivariate model.

For the multivariate analysis, ProExC and p16 staining were considered positive if they showed strong staining (score of 3), and ER, PR, vimentin, and HPV ISH were considered positive if they showed at least weak staining. A multivariate model was built on the training cases using logistic regression with the 6 markers. On the training set, the 6 marker model classified 98% (90/92) endometrial cases and 75% (12/16) endocervical cases correctly with an overall diagnostic accuracy of 94%. When applied to the test cases, the 6 marker model classified 97% (101/104) endometrial cases and 56% (10/ 18) endocervical cases correctly with an overall diagnostic accuracy of 91%. The strongest predictors of site identified by logistic regression analysis were vimentin, p16, and HPV ISH. This 3 marker model performed very similarly to the 6 marker model in the test set, correctly classifying 95% (99/104) endometrial cases and 61% (11/ 18) endocervical cases with an overall accuracy of 90%. In addition, a 3 marker panel of vimentin, p16, and ER (identified by the analysis using the R script; see below) also performed very similarly on the test set, correctly classifying 96% (100/104) endometrial cases and 44% (8/ 18) endocervical cases with an overall diagnostic accuracy of 89%.

Performance of Marker Panels

Of the single markers, HPV ISH and ProExC showed the strongest performance (87% to 88% accuracy). p16, ER, and PR showed intermediate performance (80% to 82%). Vimentin showed a small but statistically significant decrease in overall diagnostic accuracy (73%). Of the 2 marker panels, all performed best when only 1 of 2 stains was required to support endometrial origin to classify the case as endometrial, whereas both stains needed to support cervical origin to classify the case as cervical. These marker panels tended to show

comparable accuracy (85% to 93%), and overall the 2 marker panels produced a significantly higher diagnostic accuracy compared with 1 marker in isolation (3034/3435, 88% of all possible 2 marker panels vs 1126/1374, 82% of all possible 1 marker panels; $P<0.0002$). The highest diagnostic accuracy was achieved by ProExC/vimentin with a diagnostic accuracy of 93% (98% accuracy on endometrial cases and 59% accuracy on cervical cases). HPV ISH/vimentin, p16/ProExC, and HPV ISH/ProExC did not differ significantly from ProExC/vimentin with a diagnostic accuracy of 90%; accuracy for p16/vimentin was 89%.

The diagnostic accuracies of the 3 marker panels were relatively comparable with each other and ranged from 87% to 93%. Overall, the 3 marker panels showed a very small but statistically significant improved accuracy compared with the 2 marker panels (4113/4580, 89% of all possible 3 marker panels vs. 3034/3435, 88% of all possible 2 marker panels; $P=0.04$). The diagnostic accuracy of 3 marker panels consisting of (1) vimentin (2) ER or PR (3) p16 or ProExC, or (1) vimentin (2) HPV ISH (3) p16 or ProExC ranged from 89% to 93%. The 4, 5, and 6 marker panels showed comparable overall diagnostic accuracy and ranged between 88% and 92%. The overall diagnostic accuracies of the 4, 5, and 6 marker panels were not significantly different from each other or from the 3 marker panels (all $P \geq 0.3$).

Overall, these data show that the 2 and 3 marker panels tend to outperform single marker panels with a slight but statistically significant advantage to the 3 marker panels. There does not seem to be significant added benefit to using panels with more than 3 markers. The performance of 3 marker panels was comparable to the larger marker panels.

Concordance Between TMA and Whole Sections

There was good concordance between the staining patterns of the 19 whole section cases and their corresponding TMA cores for ER, PR, p16, and ProExC but not for vimentin. 4/19 (21.1%) cases were negative for vimentin on the TMA but positive on the whole sections. This conversion of a negative TMA result to positive occurred in 1/19 (5.3%) cases with ER, PR, and ProExC, and no cases with p16. The converse of a positive stain on TMA and negative on whole section occurred in 1/19 (5.3%) cases with vimentin and ER and no cases with ProExC, p16, and PR.

Although p16 showed concordant results in the above 19 cases, we found significant discrepancies between TMA and whole section results when specific cases were selected for whole section staining. 4/34 classic endocervical adenocarcinoma cases with focal strong p16 staining on TMA exhibited diffuse strong p16 reactivity on whole sections. In addition, 8/16 problem cases classified as endocervical in origin exhibited diffuse strong p16 reactivity on whole sections which was not seen on TMA cores.

Problem Cases

A total of 41 problem cases (36 TMA, 5 whole sections) were evaluated. On the basis of gross, clinical, and radiographic findings, 19 cases were determined to be endometrial in origin (18 TMA, 1 whole section) and 20 endocervical (16 TMA, 4 whole sections). Site of origin remained unknown for 2 cases. When applying the marker panels to the set of 36

TMA problem cases, the diagnostic accuracy decreased significantly. For 3 marker panels, accuracy ranged from 64% to 72%; for 2 marker panels, 61% to 69%.

Of the 19 problem cases determined to be endometrial in origin, 8 exhibited a characteristic endometrial immunoprofile: vimentin—positive, hormone receptor—positive, HPV marker—negative. 2 cases showed diffuse strong p16 reactivity, and positive vimentin and hormone receptor; these cases exhibited endometrioid morphology. Nine cases were negative for HPV markers but were also negative for both vimentin and a hormone receptor. No cases exhibited results characteristic of endocervical origin.

Of the 20 cases determined to be endocervical in origin, 13 exhibited characteristic endocervical results: vimentin—negative, hormone receptor—negative, HPV marker—positive (4 cases exhibited equivocal p16 results on TMA but were diffusely positive on whole sections). Five cases showed inconclusive results with vimentin negative and HPV marker positive (4 cases exhibited equivocal p16 results on TMA but were diffusely positive on whole sections) but hormone receptor positive. The remaining 2 cases exhibited results characteristic of endometrial origin. Determination of site of origin in 1 case was based on MRI scans showing disease limited to the cervix (hysterectomy not performed) and based on gross examination of a hysterectomy specimen in the other. In the latter case, the tumor bulk was in the cervix involving both the anterior and posterior endocervix with focal extension into the lower uterine segment. In both cases, no in situ component was identified.

For the 2 cases in which site of origin remained unclear after correlation with gross, clinical, and/or radiographic findings, the marker panel results were inconclusive and did not clearly point toward endocervical or endometrial origin.

Special Variants

Uterine serous carcinoma frequently exhibited diffuse strong reactivity for p16 (7/13, 53.8%) and ProExC (8/13, 61.5%) (Fig. 3). Fewer cases of clear cell carcinoma cases were p16 positive (2/6, 33.3%) and no cases showed diffuse strong reactivity for ProExC (0/16). HPV ISH was negative in all cases of serous and clear cell carcinomas (Table 3).

Diffuse strong p16 reactivity was seen in 50% (4/8) of minimal deviation adenocarcinomas. ProExC was positive in only 25% (2/8) of cases. Upon review of the p16 positive cases, it was noted that the p16 positive foci occurred in areas with endometrioid (Figs. 4A, B) or nonspecific cell type differentiation and not in areas with the usual mucinous morphology (Figs. 4D–F). This pattern of reactivity was not apparent with ProExC. HPV ISH was positive in 25% (1/4) of the p16 positive cases (Fig. 4C). The single cases of adenoid basal cell carcinoma (Fig. 5) and small cell carcinoma (Fig. 6) were both positive for p16, ProExC, and HPV ISH whereas the single case of mesonephric carcinoma was negative for all three stains (Table 3).

DISCUSSION

In this study, we examined a wide panel of immunohistochemical markers to determine the optimal approach for distinguishing endometrial from endocervical adenocarcinomas.

Markers that evaluate for high-risk HPV (p16, ProExC, HPV ISH) and vimentin proved to be the strongest predictors of site of origin for the usual endometrioid and mucinous subtypes of adenocarcinoma. Markers for hormone receptors (ER, PR) also performed well, but mCEA was not found to be useful. Given that the majority of endocervical adenocarcinomas are HPV-related and endometrial adenocarcinomas are not, it is not surprising that markers for HPV proved to be useful in determining site of origin. Earlier studies have established the correlation between diffuse strong reactivity for p16 and the presence of high-risk HPV integrated into the host genome in cervical dysplasia.^{19,28} However, p16 can also be overexpressed in the absence of HPV integration as exemplified by the diffuse p16 expression seen with uterine serous carcinomas. Although earlier studies have shown the use of p16 in distinguishing endocervical from endometrial primaries, no studies have examined ProExC in direct comparison with p16. Our results indicate that ProExC performs comparably with p16 in usual endometrial and endocervical adenocarcinomas. Similar to p16, ProExC is also frequently positive in uterine serous carcinomas. However, ProExC was negative in all cases of uterine clear cell carcinoma whereas p16 was positive in a third of the cases. This slight advantage of ProExC over p16 could not be further delineated given the limited number of clear cell carcinoma cases in this study.

Both the R script used to evaluate the diagnostic accuracy of all possible panels and multivariate analysis identified a 3 marker panel as optimal for determining site of origin. Using the R script, the 3 marker panel showed a small but statistically significant improvement in accuracy as compared with the 2 marker panel. However, continuing to add markers beyond the 3 marker panels was not helpful. Four-, five-, and six-marker panels did not perform better in classifying more classic or problem cases when compared with 2 or 3 marker panels. Multivariate analysis identified 3 markers—vimentin, p16 and HPV ISH—as the strongest predictors of site. The overall diagnostic accuracy of this 3 marker model (90%) was very similar to that for the 6 marker model (91%) and for a comparable 3 marker panel of vimentin, p16, and ER (89%).

Given this data, the optimal approach would be to use a 3 marker panel of vimentin, an HPV marker (p16, ProExC, or HPV ISH) and a hormone receptor marker (ER, PR). As HPV ISH is not routinely available in many pathology labs, p16 or ProExC would be the most likely choice as an HPV marker. Both ProExC and p16 require diffuse strong reactivity for the results to be interpreted as positive. Focal strong or patchy reactivity can often be seen with endometrial carcinomas and should be interpreted as negative in this context. The commercially available Ventana HPV VIII Family 16 probe and Dako HPV 16/18 probe may prove to be additionally useful markers but they were not fully evaluated in this study.

Not surprisingly, all the marker panels performed better on the classic cases than the problem cases. The diagnostic accuracy for the 3 marker panels was 89%–93% for the classic cases and 64% to 72% for the problem cases. This difference is important to note as in clinical practice immunohistochemical stains are typically obtained on problematic cases and not classic ones. Inconclusive results were exhibited by 43.9% (18/41) problem cases (11 endometrial, 5 endocervical, 2 unknown) with the 3 marker panels. Usually in these situations, more stains are performed if the initial set is inconclusive. However, our data

indicates that using more than 3 markers will not significantly improve diagnostic accuracy in problematic cases and correlation with clinical, radiographic, and gross findings will be especially important when the 3 marker panel is inconclusive. In some cases, the site will remain indeterminate even when all available information is evaluated. This was seen with 2 problem cases in which site could not be determined based on clinical, radiographic, and/or gross findings, and the 3 marker panels were inconclusive. As the optimal management for patients with tumors of indeterminate site is not known, future studies should be directed toward identification of tumors that do not neatly fit into our current oversimplified dichotomous classification system and determining the appropriate therapeutic regimens for this subset of patients.

The best panels for the usual types of endometrioid and mucinous adenocarcinoma panels did not perform as well for special variants such as uterine serous and clear cell carcinomas and minimal deviation carcinomas of the cervix. The use of ProExC and p16 in supporting endocervical origin applies primarily to the usual endometrioid and mucinous subtypes of adenocarcinomas. Although p16 reactivity can be seen in squamous morular elements of uterine endometrioid carcinomas,²² diffuse strong p16 staining is commonly seen with uterine serous carcinomas. In our study, 53.8% (7/13) serous carcinomas and 33.3% (2/6) clear cell carcinomas exhibited diffuse strong reactivity for p16. In addition, 61.5% (8/13) serous carcinomas were also positive for ProExC but no cases of clear cell carcinoma (0/6). Prior studies have reported diffuse strong p16 in uterine serous carcinomas (100%, 60/60)^{7,33} but have not commented on p16 reactivity in uterine clear cell carcinomas or on ProExC reactivity in either serous or clear cell carcinomas. As uterine serous carcinomas and clear cell carcinomas are frequently negative for vimentin and can lack hormone receptors, it is important to recognize their distinct morphology to avoid misclassification as an endocervical primary based on diffuse strong p16 reactivity or diffuse strong ProExC in cases of serous carcinoma. Other rare special variants (eg, cervical serous carcinoma, clear cell carcinoma, glassy cell carcinoma, adenoid cystic carcinoma, etc.) not represented in this study require further investigation to determine their immunohistochemical profile.

Special types of endocervical carcinomas have not been as extensively studied as the usual endometrioid and mucinous subtypes, but the limited available data indicates that certain subtypes are less frequently associated with high-risk HPV. A total of 17/19 minimal deviation endocervical adenocarcinomas and 1 case of mesonephric carcinoma were found to be HPV negative in prior studies.^{12,13,26,30,32} In contrast, all 16 cases of adenoid basal cell carcinoma have been reported to be HPV positive,^{14,26} as are the majority of cervical small cell carcinomas (HPV 94.1%, 32/34; p16 90.9%, 10/11).^{1,15,16} Although we also noted similar results for the single cases of mesonephric carcinoma, adenoid basal cell carcinoma, and small cell carcinoma in our study, we found diffuse strong p16 reactivity in 50% (4/8) and positive HPV ISH in 12.5% (1/8) of minimal deviation adenocarcinomas. This finding is higher than that reported by Mikami et al in which they found weak to strong p16 reactivity in 30% (6/20) cases of minimal deviation adenocarcinoma.²⁴ ProExC was positive in only 25% (2/8) cases. Upon review of the p16 positive cases in our study, it was noted that the p16 reactivity was predominantly limited to areas with endometrioid or nonspecific cell type differentiation and did not occur in areas with the usual mucinous morphology. In addition, HPV ISH was positive in 1 of these cases with punctuate nuclear

staining noted in an area of endometrioid differentiation. This would suggest that the subset of minimal deviation adenocarcinomas with endometrioid morphology may be more likely to be associated with high-risk HPV. On the basis of prior studies of limited numbers of cases, HPV infection is infrequent (0.07%, 1/15) in minimal deviation adenocarcinoma.^{12,13,26,30,32} Although the subtype is not noted in these studies, when photomicrographs are present, the mucinous subtype of minimal deviation adenocarcinomas is shown.¹³ Further studies, including HPV typing, of minimal deviation adenocarcinomas cases with endometrioid differentiation are needed to determine if HPV infections are more common in this subtype.

Overall, there was good concordance between the staining results for the whole sections and the TMA cores for ProExC, ER, and PR. However, vimentin was discrepant in 21% of cases (4/19) where the TMA core was negative but the whole section was positive. These discordant results may be attributed to the focality of vimentin staining which can lead to false negative results in small samples. In addition, we noted focal p16 reactivity in a subset of classic endocervical adenocarcinomas (4/34) and problem endocervical adenocarcinomas (8/16) on tissue microarray sections. To further evaluate this unexpected staining pattern, p16 was performed on corresponding whole sections and showed diffuse strong reactivity in all 12 cases. The most likely cause of this discrepancy is a mild loss of antigenicity in the TMA sections (which can occur when pre-cut TMA sections are stored at room temperature for as few as 2 days).¹¹ As this study primarily evaluated the performance of the marker panels on TMA sections, the possibility of false negative results with vimentin and p16 suggest that our results may underestimate the accuracy of the marker panels. However, as TMA cores can be viewed as a surrogate for the small samples obtained by endocervical or endometrial curettage, the possibility of false negative results should be kept in mind when evaluating vimentin or p16 results on small fragments of tumor from a curettage specimen. If equivocal results are obtained, it may be helpful to repeat the marker panel on freshly cut tissue sections or on material from a resection specimen.

One drawback to this study is the predominance of endometrial carcinoma cases. Although the relative proportion of endometrial and endocervical carcinoma cases reflect general clinical practice, it does introduce a bias toward panels seeming to perform better in identifying endometrial origin. With problematic cases, there may be morphologic features that favor either endometrial or endocervical origin but it can generally be presumed if immunohistochemical stains are being performed that there is an equal likelihood of either endometrial or endocervical origin.

In summary, 3-marker antibody panels (vimentin; ER or PR; p16 or ProExC) are optimal in evaluating cases of endometrioid and mucinous types of adenocarcinomas in which the site of origin is unclear. When these panels are inconclusive, adding additional immunohistochemical antibodies will not significantly increase diagnostic accuracy. It is important to recognize special variants such as uterine serous carcinoma and clear cell carcinoma, as these can lack vimentin expression and show extensive staining for p16 and, in cases of serous carcinoma, ProExC. As exemplified by these special variants, p16 overexpression can occur through diverse pathways, only 1 of which involves HPV integration into the host genome. When evaluating immunohistochemical results on small

biopsy or curettage samples, consideration should be given to the possibility of false negative results, especially with vimentin and p16. Given the limits of immunohistochemical stains in distinguishing between endocervical and endometrial origin, individual cases should be evaluated in the context of standard clinical, radiographic, gross, and morphologic findings.

References

1. Albores-Saavedra J, Martinez-Benitez B, Luevano E. Small cell carcinomas and large cell neuroendocrine carcinomas of the endometrium and cervix: polypoid tumors and those arising in polyps may have a favorable prognosis. *Int J Gynecol Pathol*. 2008; 27:333–339. [PubMed: 18580310]
2. Alkushi A, Irving J, Hsu F, et al. Immunoprofile of cervical and endometrial adenocarcinomas using a tissue microarray. *Virchows Arch*. 2003; 442:271–277. [PubMed: 12647218]
3. Ansari-Lari MA, Staebler A, Zaino RJ, et al. Distinction of endocervical and endometrial adenocarcinomas: immunohistochemical p16 expression correlated with human papillomavirus (HPV) DNA detection. *Am J Surg Pathol*. 2004; 28:160–167. [PubMed: 15043304]
4. Aximu D, Azad A, Ni R, et al. A pilot evaluation of a novel immunohistochemical assay for topoisomerase II-alpha and mini-chromosome maintenance protein 2 expression (ProEx C) in cervical adenocarcinoma in situ, adenocarcinoma, and benign glandular mimics. *Int J Gynecol Pathol*. 2009; 28:114–119. [PubMed: 19188825]
5. Badr RE, Walts AE, Chung F, et al. BD ProEx C: a sensitive and specific marker of HPV-associated squamous lesions of the cervix. *Am J Surg Pathol*. 2008; 32:899–906. [PubMed: 18425044]
6. Castrillon DH, Lee KR, Nucci MR. Distinction between endometrial and endocervical adenocarcinoma: an immunohistochemical study. *Int J Gynecol Pathol*. 2002; 21:4–10. [PubMed: 11781516]
7. Chiesa-Vottero AG, Malpica A, Deavers MT, et al. Immunohistochemical overexpression of p16 and p53 in uterine serous carcinoma and ovarian high-grade serous carcinoma. *Int J Gynecol Pathol*. 2007; 26:328–333. [PubMed: 17581420]
8. Conesa-Zamora P, Domenech-Peris A, Orantes-Casado FJ, et al. Effect of human papillomavirus on cell cycle-related proteins p16, Ki-67, Cyclin D1, p53, and ProEx C in precursor lesions of cervical carcinoma: a tissue microarray study. *Am J Clin Pathol*. 2009; 132:378–390. [PubMed: 19687314]
9. Conesa-Zamora P, Domenech-Peris A, Ortiz-Reina S, et al. Immunohistochemical evaluation of ProEx C in human papillomavirus- induced lesions of the cervix. *J Clin Pathol*. 2009; 62:159–162. [PubMed: 18930984]
10. Dabbs DJ, Sturtz K, Zaino RJ. The immunohistochemical discrimination of endometrioid adenocarcinomas. *Hum Pathol*. 1996; 27:172–177. [PubMed: 8617459]
11. DiVito KA, Charette LA, Rimm DL, et al. Long-term preservation of antigenicity on tissue microarrays. *Lab Invest*. 2004; 84:1071–1078. [PubMed: 15195116]
12. Ferguson AW, Svoboda-Newman SM, Frank TS. Analysis of human papillomavirus infection and molecular alterations in adenocarcinoma of the cervix. *Mod Pathol*. 1998; 11:11–18. [PubMed: 9556417]
13. Fukushima M, Shimano S, Yamakawa Y, et al. The detection of human papillomavirus (HPV) in a case of minimal deviation adenocarcinoma of the uterine cervix (adenoma malignum) using in situ hybridization. *Jpn J Clin Oncol*. 1990; 20:407–412. [PubMed: 2178197]
14. Grayson W, Taylor LF, Cooper K. Adenoid basal carcinoma of the uterine cervix: detection of integrated human papillomavirus in a rare tumor of putative “reserve cell” origin. *Int J Gynecol Pathol*. 1997; 16:307–312. [PubMed: 9421068]
15. Herrington CS, Graham D, Southern SA, et al. Loss of retinoblastoma protein expression is frequent in small cell neuroendocrine carcinoma of the cervix and is unrelated to HPV type. *Hum Pathol*. 1999; 30:906–910. [PubMed: 10452502]

16. Horn LC, Lindner K, Szepankiewicz G, et al. p16, p14, p53, and cyclin D1 expression and HPV analysis in small cell carcinomas of the uterine cervix. *Int J Gynecol Pathol.* 2006; 25:182–186. [PubMed: 16633070]
17. Ihaka R, Gentleman RR. A language for data analysis and graphics. *J Computational Graphical Statist.* 1996; 5:299–314.
18. Kamoi S, AlJuboury MI, Akin MR, et al. Immunohistochemical staining in the distinction between primary endometrial and endocervical adenocarcinomas: another viewpoint. *Int J Gynecol Pathol.* 2002; 21:217–223. [PubMed: 12068166]
19. Klaes R, Friedrich T, Spitkovsky D, et al. Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. *Int J Cancer.* 2001; 92:276–284. [PubMed: 11291057]
20. Kurman, RJ.; Norris, HJ.; Wilkinson, E. Minimal Deviation Adenocarcinoma. *Tumors of the Cervix, Vagina, and Vulva.* Washington, D.C: AFIP; 1992. p. 91-93.
21. Liu CL, Prapong W, Natkunam Y, et al. Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. *Am J Pathol.* 2002; 161:1557–1565. [PubMed: 12414504]
22. McCluggage WG, Jenkins D. p16 immunoreactivity may assist in the distinction between endometrial and endocervical adenocarcinoma. *Int J Gynecol Pathol.* 2003; 22:231–235. [PubMed: 12819388]
23. McCluggage WG, Sumathi VP, McBride HA, et al. A panel of immunohistochemical stains, including carcinoembryonic antigen, vimentin, and estrogen receptor, aids the distinction between primary endometrial and endocervical adenocarcinomas. *Int J Gynecol Pathol.* 2002; 21:11–15. [PubMed: 11781517]
24. Mikami Y, Kiyokawa T, Hata S, et al. Gastrointestinal immunophenotype in adenocarcinomas of the uterine cervix and related glandular lesions: a possible link between lobular endocervical glandular hyperplasia/pyloric gland metaplasia and ‘adenoma malignum’. *Mod Pathol.* 2004; 17:962–972. [PubMed: 15143335]
25. Pinto AP, Schlecht NF, Woo TY, et al. Biomarker (ProEx C, p16(INK4A), and MiB-1) distinction of high-grade squamous intraepithelial lesion from its mimics. *Mod Pathol.* 2008; 21:1067–1074. [PubMed: 18552822]
26. Pirog EC, Kleter B, Olgac S, et al. Prevalence of human papillomavirus DNA in different histological subtypes of cervical adenocarcinoma. *Am J Pathol.* 2000; 157:1055–1062. [PubMed: 11021808]
27. Riethdorf L, Riethdorf S, Lee KR, et al. Human papillomaviruses, expression of p16, and early endocervical glandular neoplasia. *Hum Pathol.* 2002; 33:899–904. [PubMed: 12378514]
28. Sano T, Oyama T, Kashiwabara K, et al. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. *Am J Pathol.* 1998; 153:1741–1748. [PubMed: 9846965]
29. Staebler A, Sherman ME, Zaino RJ, et al. Hormone receptor immunohistochemistry and human papillomavirus in situ hybridization are useful for distinguishing endocervical and endometrial adenocarcinomas. *Am J Surg Pathol.* 2002; 26:998–1006. [PubMed: 12170086]
30. Toki T, Zhai YL, Park JS, et al. Infrequent occurrence of high-risk human papillomavirus and of p53 mutation in minimal deviation adenocarcinoma of the cervix. *Int J Gynecol Pathol.* 1999; 18:215–219. [PubMed: 12090589]
31. Walts AE, Bose S. p16, Ki-67, and BD ProExC immunostaining: a practical approach for diagnosis of cervical intraepithelial neoplasia. *Hum Pathol.* 2009; 40:957–964. [PubMed: 19269017]
32. Xu JY, Hashi A, Kondo T, et al. Absence of human papillomavirus infection in minimal deviation adenocarcinoma and lobular endocervical glandular hyperplasia. *Int J Gynecol Pathol.* 2005; 24:296–302. [PubMed: 15968208]
33. Yemelyanova A, Ji H, Shih IM, et al. Utility of p16 expression for distinction of uterine serous carcinomas from endometrial endometrioid and endocervical adenocarcinomas: immunohistochemical analysis of 201 cases. *Am J Surg Pathol.* 2009; 33:1504–1514. [PubMed: 19623034]

34. Yemelyanova A, Vang R, Seidman JD, et al. Endocervical adenocarcinomas with prominent endometrial or endomyometrial involvement simulating primary endometrial carcinomas: utility of HPV DNA detection and immunohistochemical expression of p16 and hormone receptors to confirm the cervical origin of the corpus tumor. *Am J Surg Pathol*. 2009; 33:914–924. [PubMed: 19295407]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

FIGURE 1. Endometrial adenocarcinoma, endometrioid type (A) with focal strong p16 (B), focal strong ProExC (C), positive vimentin (D), positive estrogen receptor (E), positive progesterone receptor (F); negative HPV ISH (not shown).

FIGURE 2. Endocervical adenocarcinoma (A) with diffuse strong p16 (B), diffuse strong ProExC (C), positive HPV ISH (D), negative vimentin—stromal staining only (E), negative estrogen receptor (F); negative progesterone receptor (not shown).

FIGURE 3.

Uterine serous carcinoma (A) with diffuse strong p16 (B) and diffuse strong ProExC (C) staining. Uterine clear cell carcinoma (D) with diffuse strong p16 (E) and focal strong (interpreted as negative) ProExC (F) staining.

FIGURE 4. Endocervical minimal deviation adenocarcinoma, endometrioid type (A) with diffuse strong p16 (B) and positive HPV ISH (C); mucinous type (D) with negative p16 (E) and negative ProExC (F).

FIGURE 5. Adenoid-basal cell carcinoma (A) with diffuse strong p16 (B) and diffuse strong ProExC (C).

FIGURE 6.
Small cell carcinoma (A) with diffuse strong p16 (B) and diffuse strong ProExC (C).

TABLE 1
Review of Literature: Results of Marker Panels for Distinguishing Endometrial From Endocervical Primary (Usual Types)

	Endometrial						Endocervical					
	p16	HPV ISH	ER	PR	VIM	CEA	p16	HPV ISH	ER	PR	VIM	CEA
Yemelyanova, et al ³⁰	3.0% (3/101)	—	—	—	—	—	100% (51/51)	84.3% (43/51)	—	—	—	—
Ansari-Lari, et al ³	0% (0/24)	0% (0/24)	—	—	—	—	94.7% (18/19)	73.7% (14/19)	—	—	—	—
Alkushi, et al ²	—	—	72.5% (37/51)	—	90.6% (48/53)	17.3% (9/52)	—	—	16.3% (8/49)	—	7.7% (4/52)	30.8% (16/52)
McCluggage, et al ²²	10.3% (3/29)	—	—	—	—	—	95.7% (22/23)	—	—	—	—	—
Plunkett, 2003	—	2% (1/50)	—	—	—	—	—	78% (39/50)	—	—	—	—
Kamoi, et al ¹⁸	—	—	65.9% (29/44)	70.5% (31/44)	61.4% (27/44)	6.8% (3/44)	—	—	12.5% (2/16)	25.0% (4/16)	12.5% (2/16)	37.5% (6/16)
Staebler, et al ²⁹	—	0% (0/24)	75% (18/24)	95.8% (23/24)	—	—	—	66.7% (16/24)	4.2% (1/24)	4.2% (1/24)	—	—
Castrillon, 2001	—	—	—	—	96.7% (29/30)	26.7% (8/30)	—	—	—	—	6.9% (2/29)	62.1% (18/29)
McCluggage, et al ²²	—	—	93.3% (28/30)	—	96.7% (29/30)	70% (21/30) [*]	—	—	38.5% (10/26)	—	7.7% (2/26)	96.2% (25/26)
Dabbs, et al ¹⁰	—	—	—	—	81.5% (22/27)	0% (0/27)	—	—	—	—	13.0% (3/23)	95.7% (22/23)

* Predominantly staining of benign squamous components.

CEA indicates monoclonal carcinoembryonic antigen; ER, estrogen receptor; HPV, human papilloma virus; ISH, in situ hybridization; PR, progesterone receptor; VIM, vimentin.

TABLE 2**Antibody Reagents and Conditions**

Antibody	Manufacturer	Clone	Dilution	Pre-treatment
ER	Dako	1D5	1:80	Citrate
PR	Dako	PgR 636	1:80	Citrate
Vimentin	Dako	Vim 3B4	1:200	Citrate
CEA	Lab Vision	COL-1	1:20	None
p16	mtm Laboratories	E6H4	Predilute	Tris buffer (pH9)
ProExC	BD	MCM2 26H6.19, MCM2 27C5.6, TOP2A SWT3D1	Predilute	EDTA
HPV ISH	Ventana		Predilute	Ventana protease 3

CEA indicates monoclonal carcinoembryonic antigen; ER, estrogen receptor; HPV, human papilloma virus; ISH, in situ hybridization; PR, progesterone receptor.

TABLE 3

Special Types of Endometrial and Endocervical Carcinoma

Carcinoma Type	ER	PR	Vimentin	p16	ProEx C	HPV ISH
Uterine serous carcinoma	38.5% (5/13)	15.4% (2/13)	15.4% (2/13)	53.8% (7/13)	61.5% (8/13)	0% (0/11)
Uterine clear cell carcinoma	66.7% (4/6)	50% (3/6)	33.3% (2/6)	33.3% (2/6)	0% (0/6)	0% (0/6)
Minimal deviation endocervical adenocarcinoma	12.5% (1/8)	25% (2/8)	12.5% (1/8)	50% (4/8)	25% (2/8)	12.5% (1/8)
Cervical small cell carcinoma	0% (0/1)	0% (0/1)	0% (0/1)	100% (1/1)	100% (1/1)	100% (1/1)
Mesonephric carcinoma	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)
Cervical adenoid basal cell carcinoma	0% (0/1)	0% (0/1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)

ER indicates estrogen receptor; HPV, human papilloma virus; ISH, in situ hybridization; PR, progesterone receptor.