Gynecologic Oncology Reports 33 (2020) 100614

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor

Case series

Novel immunohistochemical markers in the differential diagnosis of endocervical and endometrial adenocarcinoma: The added benefit of CAIX and PAX8

Ana I. Hernandez-Caballero[a,*], Koah R. Vierkoetter[a], Hyeong Jun Ahn[b], David Shimizu[a], Keith Terada[c]

[a] University of Hawaii, John A. Burns School of Medicine, Department of Pathology, 1301 Punchbowl Street, Honolulu 96813, HI, USA
[b] University of Hawaii, John A. Burns School of Medicine, Department of Quantitative Health Sciences, 651 Ilalo Street, Medical Education Building, Suite 411, Honolulu 96813, HI, USA
[c] University of Hawaii, John A. Burns School of Medicine, Department of Obstetrics, Gynecology and Women's Health, 1301 Punchbowl Street, Honolulu 96813, HI, USA

ARTICLE INFO

Keywords:
Endometrial adenocarcinoma
Endocervical adenocarcinoma
CAIX
PAX8
Immunohistochemistry

ABSTRACT

In a biopsy specimen, adenocarcinomas of the endometrium and uterine cervix may demonstrate significant morphologic overlap. The distinction between these two entities prior to surgical resection is clinically significant as assigning the primary site dictates treatment and prognosis. This diagnostic dilemma is approached by the application of a panel of immunohistochemical stains, traditionally composed of CEA, vimentin, p16, ER, and PR. Most cases are successfully managed with this panel; however, in difficult cases additional tools are needed to suggest a more definitive diagnosis. In this study, we reviewed the efficacy of the customary panel of stains, as well as the added value of new stains in the diagnosis of endocervical adenocarcinoma. Our cohort included biopsy samples of 90 patients (81 endometrial and 9 endocervical adenocarcinomas) with a subsequent hysterectomy for confirmation of diagnosis. This study validated the customary panel of stains and suggests additional markers to aid in the differential diagnosis (PAX8 and CAIX). The addition of PAX8 to the traditional panel increases PPV from 85.71% to 100%. A PPV of 100% may also be attained with fewer stains (five total), with the application of a proposed new panel, which includes PAX8, CAIX, CEA, p16 and ER. This is the first-time differential expression of CAIX has been suggested in the distinction between endocervical and endometrial adenocarcinomas.

1. Introduction

In a biopsy specimen, adenocarcinomas of the endometrium and uterine cervix may demonstrate significant morphologic overlap. Both entities demonstrate variable degrees of glandular differentiation, with glands lined by columnar epithelium, round to ovoid nuclei, mildly coarse chromatin and intracytoplasmic mucin. Squamous metaplasia, conventionally thought to support endometrial origin in glandular proliferations, can also be encountered in endocervical adenocarcinomas (Hirschowitz et al., 2007). Although in a resection specimen, such as a cone biopsy or hysterectomy, anatomic location is key in assigning the primary site, the differential diagnosis may be particularly challenging in biopsies or curettage specimens where it is not uncommon to sample endocervical tissue “en-route” to the endometrium.

The distinction between these two entities prior to surgical resection is clinically significant as assigning the primary site dictates treatment; depending on clinical stage, endometrial and endocervical carcinomas have different preoperative management and surgical methods. Generally, endometrial cancer is managed surgically with adjuvant therapy offered depending on features of the tumor. This is in contrast to endocervical cancer, where patients may be offered radiation therapy alone in select cases. Prognostically, the malignancies also differ; while low-grade endometrial cancers tend to have a good prognosis (5-year survival rate of about 95%) (Gottwald et al., 2010), endocervical adenocarcinomas carry a poor prognosis at advanced stage (5-year survival rate of about 84%) (Takeuchi, 2016).

Usually, this diagnostic dilemma is approached by the application of a panel of immunohistochemical stains, which, depending on the case at hand, may consist of a traditional expanded panel or focused “lean” group of markers. The literature has demonstrated that

* Corresponding author.
E-mail addresses: ana21@hawaii.edu (A.I. Hernandez-Caballero), koah@hawaii.edu (K.R. Vierkoetter), hjahn@hawaii.edu (H.J. Ahn), dshimizu@queens.org (D. Shimizu), teradake@hawaii.edu (K. Terada).

https://doi.org/10.1016/j.gore.2020.100614
Received 2 June 2020; Received in revised form 20 July 2020; Accepted 22 July 2020
Available online 27 July 2020
2352-5789/ © 2020 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

carcinoembryonic antigen (CEA) and p16 are markers of endocervical origin, whereas vimentin, estrogen receptor (ER) and progesterone receptor (PR) favor endometrial origin (Castrillon et al., 2002; Kamoi et al., 2002). Most cases are successfully managed with a limited panel; however, in difficult cases additional tools are needed to suggest a more definitive diagnosis. In this study, we reviewed the efficacy of the customary panel of stains, as well as the added value of new stains, in the diagnosis of endocervical adenocarcinoma in biopsy samples of 90 patients with a subsequent hysterectomy.

2. Material and methods

The study material consisted of selected slides and tissue blocks from 90 biopsy specimens with a subsequent hysterectomy for confirmation of diagnosis retrieved from the archives of the Queen's Medical Center Department of Pathology, Honolulu, Hawaii accessioned over a 9-year period. Eighty-one cases of endometrial adenocarcinoma and 9 endocervical adenocarcinomas were included. Cases without residual carcinoma in the hysterectomy specimen were excluded. Institutional ethics committee approval was obtained for this retrospective immunohistochemical study.

All hematoxylin and eosin (H&E) stained slides from each case were reviewed by two gynecologic pathologists (KV and DS), and a slide with representative tumor selected from each case. Formalin fixed tissue microarrays (TMAs) were constructed from 2.0 mm representative areas of tumor and evaluated using immunohistochemistry (IHC) staining with antibodies for CEA, Vimentin, p16, ER, PR, PAX-2, PAX-8, CAIX, Arid1a, PTEN, and HNF1b.

Four μm tissue sections from the TMA were stained with H&E, to assess adequacy, with further sections stained with the selected panel of antibodies. Antigen retrieval was performed with EnVision FLEX Target Retrieval Solution (Dako, Santa Clara, CA) at 97-C for 20 min. Protein expression was evaluated using antibodies to CEA (clone II-7, ready to use (RTU) dilution, Leica), Vimentin (clone V9, RTU dilution, Agilent), p16 (clone ink4a, RTU dilution, ENZO), ER (clone EP1, RTU dilution, Agilent), PR (clone PgR636, RTU dilution, Agilent), Pax-2 (EP235, RTU dilution, Cell Marque), PAX-8 (clone MRQ-50, 1:100 dilution, Cell Marque), CAIX (clone TH22, 1:100 dilution, Leica), ARID1A (clone EPR13501, 1:500 dilution, Abcam), PTEN (clone 6H2.1, 1:100 dilution, Dako), and HNF1b (polyclonal, 1:1 dilution, Sigma) on 4 μm tissue sections. Detection was achieved using the bond polymer refine detection kit (Leica Biosystems, Buffalo Grove, IL). Diaminobenzidine (Dako) and Hematoxylin (Dako) were used for chromogenic detection and counter staining, respectively.

Demographic and clinical characteristics were summarized by mean and standard deviation (SD) for continuous variable and frequencies with percentages for categorical variables. The variables were compared between endocervical and endometrial adenocarcinoma group by two sample *t* test for age and Fisher's exact test for categorical variables. Positive predictive value (PPV) and negative predictive values (NPV) with 95% confidence interval were calculated for the identification of endocervical adenocarcinoma using different type of diagnostic panels. The comparison of PPV and NPV measures between the different diagnostic panels was assessed using generalized score statistic (Leisenring et al., 2000). All the analyses were conducted using SAS version 9.4 (SAS Institute, Cary North Carolina) and p-value of less than 0.05 was considered statistically significant.

3. Results

The average age at diagnosis was 58 years (SD = 11.65). Patient population and tumor characteristics are summarized in Table 1. Immunohistochemical findings are displayed in Table 2. A significant difference in immunostaining pattens between endocervical and endometrial carcinomas was demonstrated with CEA ($p = 0.008$), vimentin ($p = 0.002$), p16 ($p = 0.001$), ER ($p < 0.001$), PR ($p = 0.001$), PAX-8 ($p = 0.013$), and CAIX ($p = 0.04$). Endometrial adenocarcinoma more frequently expressed PAX-8, CAIX, vimentin, PR and ER antigens; CEA and p16 were more frequently positive in endocervical adenocarcinoma.

Table 1
Patient demographics and tumor characteristics.

	Endocervical adenocarcinoma (n = 9)	Endometrial adenocarcinoma (n = 81)
Age at diagnosis	54.8 years (SD = 19.21)	60.9 years (SD = 10.50)
Histologic type (hysterectomy)	Endocervical adenocarcinoma, usual type (8; 88.9%) Villoglandular (1; 11.1%)	Endometrioid (75; 92.6%) Serous (1; 1.3%) Mucinous (4; 4.8%) Mixed (1; 1.3%)
Histologic grade (hysterectomy)	Grade 1 (2; 22.2%) Grade 2 (7; 77.8%) Grade 3 (none)	Grade 1 (50; 61.7%) Grade 2 (17; 21%) Grade 3 (14; 17.3%)
Stage at final diagnosis (hysterectomy)	Stage I (6; 66.7%) Stage II (3; 33.3%)	Stage I (65; 80.3%) Stage II (5; 6.1%) Stage III (10; 12.3%) Stage IV (1; 1.3%)

Table 2
Comparison of endocervical adenocarcinoma and endometrial adenocarcinoma immunohistochemical staining patterns.

	Endocervical Adenocarcinoma (n = 9)	Endometrial Adenocarcinoma (n = 81)	p value
CEA	77.8% (7/9)	30.9% (25/81)	0.008*
Vimentin	11.1% (1/9)	76.5% (62/81)	0.002*
P16	77.8% (7/9)	13.6% (11/81)	0.001*
ER	11.1% (1/9)	92.6% (75/81)	< 0.001*
PR	11.1 (1/9)	77.8% (63/81)	0.001*
PAX-2	11.1% (1/9)	13.6% (11/81)	0.99
PAX-8	66.7% (6/9)	96.3% (78/81)	0.013*
CAIX	11.1% (1/9)	48.2% (39/81)	0.039*
Arid1a	88.9% (8/9)	70.4% (57/81)	0.435
PTEN	66.7% (6/9)	45.7% (37/81)	0.301
HNF1b	11.1% (1/9)	75.3% (61/81)	0.69

* Statistically significant where p value < 0.05.

Five diagnostic panels were studied for the detection of endocervical adenocarcinoma. These included an expanded "Traditional Panel" (CEA, vimentin, p16, ER and PR) and the Traditional Panel plus PAX8. Two versions of frequently used focused panels, deemed "Lean" were also examined, Lean Panel 1 (vimentin, p16 and ER) and Lean Panel 2 (CEA, p16 and ER). A novel panel, "New Panel," (CEA, p16, ER, PAX8 and CAIX) was also examined. The positive predictive value (PPV), negative predictive value (NPV) and 95% confidence intervals of the panels are shown in Table 3.

4. Discussion

The preoperative distinction between endometrial and endocervical adenocarcinomas is important as low stage endometrial adenocarcinoma is treated by simple hysterectomy, while management of cervical adenocarcinoma includes radiotherapy with or without radical hysterectomy (Takeuchi, 2016; National Comprehensive Cancer Network, 2020). Survival rates also differ between these two sites. Whereas low-grade endometrial cancers tend to have a good prognosis (5-year survival rate of about 95%) (Gottwald et al., 2010), endocervical adenocarcinomas carry a poor prognosis at advanced stage (5-year survival

Table 3
Positive and negative predictive values of panels used in the identification of endocervical adenocarcinoma.

Panel	PPV	95% CI		NPV	95% CI	
		Lower Limit	Upper Limit		Lower Limit	Upper Limit
Traditional	85.71	0.42	0.99	96.39	0.89	0.99
Traditional plus PAX8	100	0.15	1.00	92.05	0.84	0.96
Lean 1	77.78	0.39	0.97	97.53	0.91	0.99
Lean 2	85.71	0.42	0.99	96.39	0.89	0.99
New Panel	100	0.15	1.00	92.05	0.84	0.96

PPV: positive predictive value. NPV: negative predictive value. CI: confidence interval.
Traditional Panel: CEA, vimentin, p16, ER and PR; Traditional Panel plus PAX8: CEA, vimentin, p16, ER, PR and PAX8; Lean 1: vimentin, p16 and ER; Lean 2: CEA, p16 and ER; New Panel: CEA, p16, ER, PAX8 and CAIX.

rate of about 84%) (Takeuchi, 2016). Although the overall incidence of cervical cancer is declining, the incidence of endocervical adenocarcinoma is on the rise. Smith et al. (2000) report a 29.1% age-adjusted increase incidence in cervical adenocarcinoma using the SEER database. Given increasing incidence of endocervical adenocarcinoma, the diagnostic challenge of differentiating endocervical versus endometrial origin in biopsy specimens remains clinically relevant.

We studied 90 cases of endocervical and endometrial adenocarcinoma using tissue microarray and multiple antibodies, confirming the utility of recognized immunohistochemical markers and identifying two additional stains, PAX8 and CAIX, to apply when faced with morphologically challenging cases. In practice, pathologists may apply a full traditional panel of immunostains up front, or alternately a focused "lean" panel. In difficult cases, even when a full battery of markers is applied, a precise anatomic diagnosis may not be attained. This study validated the customary panel of stains in the diagnosis of endocervical adenocarcinoma, with the suggestion of additional stains to add to clarify the differential diagnosis – PAX8 and CAIX.

One of the two novel markers, PAX-8, is a marker frequently utilized in identifying gynecologic tract malignancies (Yemelyanova et al., 2014). In the present study, PAX8 was expressed in 66.7% of endocervical adenocarcinomas and 96.3% of endometrial adenocarcinomas. Although the marker achieved statistically significance ($p = 0.012$) in our analysis, other studies have failed to demonstrate this association. Liang et al. (2016) compared 26 cases of endocervical adenocarcinoma and 20 cases of endometrial adenocarcinoma and found that all endometrial endometrioid carcinomas expressed PAX8, while 81% (21/26) cases of endocervical adenocarcinoma were also positive for the marker. However, their analysis included diverse endocervical adenocarcinoma histotypes, described as usual, endometrioid and poorly differentiated. This is in contrast to the current study which included only usual type and villoglandular histologies, with no high grade endocervical tumors.

The second novel marker, CAIX, functions in the adaptation of tumor cells to hypoxic conditions. Tumorgenesis related to CAIX is linked to Hypoxia-inducible factor-1α (HIF-1α). Authors have demonstrated that expression of HIF-1α and downstream genes Glut-1, VEGF and CAIX, promotes angiogenesis in Type 1 endometrial cancers, with CAIX expression in up to 92.3% of endometrial endometrioid adenocarcinomas (Horrée et al., 2007). In the current study, CAIX was expressed in 11.1% of endocervical adenocarcinomas and 48.2% of endometrial adenocarcinomas, a difference that reached statistical significance ($p = 0.039$). To our knowledge, this is the first time this relationship has been established.

In addition, we studied other immunostains with known expression in gynecologic malignancies for possible distinction between endocervical and endometrial primary sites of origin. However, PAX-2, Arid1a, PTEN, and HNF1b did not show significant differential expression between adenocarcinomas of the two sites. These findings could be explained by biologic differences or could be due to the small sample size. Further studies are needed to further clarify the usefulness of these markers.

Our results show that a traditional panel comprised of CEA, vimentin, p16, ER and PR has a PPV of 85.71% in the diagnosis of endocervical adenocarcinoma. This is comparable to the use of a focused "lean panels" that include CEA, p16 and ER (Lean panel 2) or vimentin, p16 and ER (Lean panel 1); showing PPVs of 85.71% and 77.78% respectively. We showed that while the addition of PAX8 to the traditional panel for a total of six stains increases PPV to 100%, a 100% PPV may also be attained with fewer stains (five total), as we observed in the "New Panel", which includes PAX8 and CAIX. Although more studies are needed to support our findings, this data suggests that the "New panel" offers a diagnostic advantage over the traditional panel. While the PPV of the traditional panel is 85.71%, itself a high value, there is an almost 15% margin of error compared to the "New panel," which reaches a PPV of 100%. Thus, in our cohort, the PPV of the "New panel" is higher than that of the traditional panel. Based on our sample size and power analysis, we will need at least 75 endocervical cases to detect the difference to achieve 90% power.

This is the first-time differential expression of CAIX has been suggested in the distinction between endocervical and endometrial adenocarcinomas, including as part of a panel including CEA, p16, ER, PAX8 and CAIX. This study was limited by relatively fewer endocervical adenocarcinomas in the cohort, with 9 endocervical adenocarcinoma compared to 81 endometrial cases. This is reflective of population incidences of these tumor types. In addition, our study excluded cases without a subsequent hysterectomy specimen for confirmation of diagnosis, which is ultimately a strength when determining site of origin. Additional studies are needed to further validate these findings in larger cohorts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding acknowledgement

Hyeong Jun Ahn, PhD, is partially supported by the National Institute of Health (U54MD00760131 and U54GM104944). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

This study is supported by The Kosasa Endowment by the University of Hawaii. The content is solely the responsibility of the authors and does not necessarily represent the official views of the UH.

References

Castrillon, D.H., Lee, K.R., Nucci, M.R., 2002. Distinction between endometrial and endocervical adenocarcinoma: an immunohistochemical study. Int. J. GynecolPathol. 21, 4–10.
Gottwald, L., Pluta, P., Piekarski, J., et al., 2010. Long-term survival of endometrioid endometrial cancer patients. Arch. Med. Sci. 6 (6), 937–944.
Hirschowitz, L., Sen, C., Murdoch, J., 2007. Primary endometrioid adenocarcinoma of the cervix with widespread squamous metaplasia – a potential diagnostic pitfall. Diagn. Pathol. 2, 40.
Horrée, N., van Diest, P.J., van der Groep, P., et al., 2007. Hypoxia and angiogenesis in endometrioid endometrial carcinogenesis. Cell. Oncol. 29 (3), 219–227.
Kamoi, S., AlJuboury, M.I., Akin, M.R., et al., 2002. Immunohistochemical staining in the distinction between primary endometrial and endocervical adenocarcinomas:

another viewpoint. Int. J. GynecolPathol. 21, 217–223.
Leisenring, W., Alonzo, T., Pepe, M.S., 2000. Comparisons of predictive values of binary medical diagnostic tests for paired designs. Biometrics 56 (2), 345–351.
Liang, L., Zheng, W., Liu, J., Liang, S.X., 2016. Assessment of the utility of PAX8 immunohistochemical stain in diagnosing endocervical glandular lesions. Arch. Pathol. Lab Med. 140 (2), 148–152.
National Comprehensive Cancer Network, 2020. Uterine neoplasms. Retrieved from https://www.nccn.org/professionals/physician_gls/default.aspx.
Smith, H.O., Tiffany, M.F., Qualls, C.R., 2000. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States–a 24-year population-based study. Gynecol. Oncol. 78 (2), 97–105.
Takeuchi, S., 2016. Biology and treatment of cervical adenocarcinoma. Chin. J. Cancer Res. 28 (2), 254–262.
Yemelyanova, A., Gown, A.M., Wu, L.S.F., et al., 2014. PAX8 expression in uterine adenocarcinomas and mesonephric proliferations. Int. J. GynecolPathol. 33 (5), 492–499.