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Immunophenotypic diversity of endometrial adenocarcinomas: implications for differential diagnosis

Michelle Reid-Nicholson[1,*,**], Pratibha Iyengar[1,**,†], Amanda J Hummer[2], Irina Linkov[3], Marina Asher[1] and Robert A Soslow[1]

[1]*Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA;* [2]*Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA and* [3]*Immunohistochemistry Core Laboratory, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

Many endometrial adenocarcinomas, particularly those of endometrioid type, express estrogen receptors (ERs), progesterone receptors (PRs), and vimentin. This typical immunophenotype is frequently considered a standard against which others are compared when immunohistochemistry is used for differential diagnosis. We tested large numbers of endometrial cancers, enriched for high-grade tumors, to determine whether this reported immunophenotype was valid and whether expression differences between types of endometrial carcinoma could be exploited for diagnostic purposes. Immunohistochemical stains were performed on the following types of endometrial cancers using established methodology: International Federation of Gynecology and Obstetrics (FIGO) grades 1 and 2 endometrioid—42; FIGO grade 3 endometrioid—40; serous—24; clear cell—11; carcinosarcoma—9. In total, 92% of serous carcinomas expressed p16 strongly compared to weak-to-moderate expression of p16 in 7–67% of other tumors (FIGO grades 1 and 2 carcinoma and carcinosarcoma, respectively). A total of 84% of FIGO grades 1 and 2 carcinomas expressed ER compared to 9–54% of other tumors (clear cell and serous carcinomas respectively); 83% of FIGO grades 1 and 2 expressed PR compared to 11–54% of other carcinomas (carcinosarcoma and serous carcinoma, respectively). Most carcinomas were negative for monoclonal carcinoembryonic antigen (mCEA), and those that were positive showed mostly only focal membrane expression. Vimentin was expressed in nearly every tumor. Most tumors were diffusely vimentin positive, but a large range of expression patterns, from focal to diffuse and from weak to strong, was noted. Only 70% of FIGO grades 1 and 2 endometrioid carcinomas and 26% of grade 3 endometrioid carcinomas possessed the reportedly characteristic endometrial cancer immunophenotype p16 (−), ER (+), PR (+), mCEA (−), and vimentin (+). Endometrial cancers demonstrate substantial immunophenotypic diversity that remained apparent even within groups of similar histologic subtype and grade. ER, PR, and p16 expression was more illustrative of tumor type and degree of differentiation than they were of endometrial origin. In contrast, the vimentin-positive/CEA-negative phenotype remained the most constant among all endometrial cancers.
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It remains a diagnostic challenge to discriminate between some endometrial and endocervical adenocarcinomas, especially in curettage material. Morphologically similar carcinomas, such as endocervical adenocarcinomas, have been reported to express p16[1–3] and carcinoembryonic antigen (CEA),[4–6] whereas endometrial adenocarcinomas frequently show estrogen receptor (ER),[7,8] progesterone receptor (PR),[7,8] and vimentin expression.[4,7,9] In practice, many pathologists have used immunohistochemical panels composed of markers against these antigens for determining site of origin. In most cases, this approach is not problematic because endometrioid adenocarcinomas usually derive from the uterine corpus. However, there is substantial morphologic heterogeneity within each tumor family related to types and degree of differentiation, which usually means that there exists considerable

Correspondence: Dr RA Soslow, MD, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, C524, New York, NY 10021, USA.
E-mail: soslowr@mskcc.org
*Current address: Department of Pathology, Medical College of Georgia, Augasta, GA, USA.
**Both authors contributed equally to this work and are considered co-first authors.
†Current address: Department of Laboratory Medicine, Credit Valley Hospital, Mississauga, ON, Canada.
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immunophenotypic variation within each tumor family. Another problem with using this approach is that there are several different tumor types other than endometrioid adenocarcinoma that arise in the endometrium, potentially confusing site assessment. We therefore hypothesized that an immunohistochemical profile is likely to be informative about tumor type and degree of differentiation, but not necessarily about site of origin. We tested large numbers of endometrial cancers, enriched for high-grade tumors, to determine whether the ER/PR/vimentin-positive, p16/CEA-negative immunophenotype was valid and whether expression differences between types of endometrial carcinoma could be exploited for diagnostic purposes.

Materials and methods

Immunohistochemical stains were performed on the following types of endometrial cancers using established methodology: International Federation of Gynecology and Obstetrics (FIGO) grades 1 and 2 endometrioid carcinomas, 42; FIGO grade 3 endometrioid carcinomas, 40; serous carcinomas, 24; clear cell carcinomas, 11; and carcinosarcoma, 9.

Study Population

Patients undergoing surgery for gynecologic malignancies at Memorial Sloan-Kettering Cancer Center (MSKCC) had their tumor specimens banked under an Institutional Review Board (IRB)-approved tissue acquisition protocol after giving their informed consent. Tumor microarrays were constructed using micro-core hysterectomy tumor specimens from patients whose tissues were selected to provide a broad representation of types, grades, and stages of endometrial cancers. Informative immunohistochemistry data from the microarray were available for 97 patients, including 42 with FIGO grades 1 and 2 endometrioid carcinomas, 36 with FIGO grade 3 endometrioid carcinoma, four with serous carcinoma, six with clear cell carcinoma, and nine with carcinosarcoma. In an effort to expand the numbers of tumor types that were poorly represented in the microarray, we randomly selected from the surgical pathology files at MSKCC additional recent cases of endometrial serous carcinoma ($n=20$), clear cell carcinoma ($n=5$), and FIGO grade 3 endometrioid adenocarcinoma ($n=4$).

Pathology Review

All tumor slides were reviewed by at least two reference pathologists. We confirmed endometrial origin, histologic subtype and grade, where applicable, of every tumor studied with immunohistochemistry.

Tumor Microarrays

Core needle biopsies of pre-existing paraffin-embedded tissue were obtained and then re-embedded in an arrayed master block using techniques originally developed by Kononen *et al*[10] and then modified by Hedvat *et al*[11] We used the Beecher Instruments (Sun Prairie, WI, USA) arraying device to produce sample circular spots that were 0.6 mm in diameter. Three core needle specimens were obtained from each tumor and companion tissue specimen.

Immunohistochemistry

Immunohistochemistry was performed according to standard protocols (Table 1). We used an immunohistochemical scoring system that took into account distribution and intensity of immunoreactivity. 1+ results were characterized by focal staining of weak intensity. 2+ results showed either diffuse weak staining or focal/patchy moderately intense staining. 3+ cases demonstrated either diffuse moderately intense staining or focal/patchy intense staining. 4+ cases were diffusely and strongly immunoreactive. Only the epithelial component of carcinosarcomas was scored. Scores of 1+ and 2+ are described in the manuscript as 'weak-to-moderate' and scores of 3+ and 4+ are described as 'strong.'

Table 1 Immunohistochemistry material and methods

	Clone	*Vendor*	*Location*	*Antigen retrieval*[a]	*Dilution*	*Pattern*
p16	16P04	Neo Markers/Lab Vision	Fremont, CA	Citrate, pH 6.00	1:400	C, N
ER	ERID5	Beckman Coulter	Miami, FL	Citrate, pH 6.00	1:100	N
PR	10A9	Beckman Coulter	Miami, FL	—	1:200	N
mCEA	A5B7	DAKO	Carpinteria, CA	Citrate, pH 6.00	1:500	C, M
Vimentin	V9	DAKO	Carpinteria, CA	Citrate, pH 6.00	1:4000	C

ER, estrogen receptor; PR, progesterone receptor; mCEA, monoclonal carcinoembryonic antigen; C, cytoplamsic; N, nuclear; M, membrane.
[a]Heat-induced antigen retrieval using a microwave.

Statistical Evaluation

Immunohistochemical results were considered negative if the score was no higher than 0. Stains were considered positive if the score was 1 or higher. The chi-square test was used to determine a significant difference in histologic subtype and marker expression for p16, ER, PR, CEA, and vimentin.

Results

Detailed immunohistochemical results are presented in Table 2. Summaries of these data follow.

p16 (Figures 1 and 2): p16 was largely negative in FIGO grades 1 and 2 endometrioid adenocarcinomas, while 25% of FIGO grade 3 tumors were positive. In all, 92% of serous carcinomas were p16-positive, and 45% of clear cell carcinomas expressed p16. A total of 67% of carcinosarcomas were positive. Tumors showing the strongest immunoreactivity were serous carcinomas and the epithelial component of carcinosarcomas. With the exception of approximately one-third of clear cell carcinomas, which were strongly labeled, all of the remaining positive tumors were weakly or moderately immunoreactive. Tumor cells demonstrating immunoreactivity showed cytoplasmic and nuclear decoration.

ER and PR (Figures 3–5): In all, 84% of FIGO grades 1 and 2 endometrioid carcinomas expressed ER compared to 50% of FIGO grade 3 carcinomas, 54% of serous carcinomas, and 9% of clear cell carcinomas. A total of of FIGO grades 1 and 2 endometrioid carcinomas expressed PR compared to 42% of FIGO grade 3 carcinomas, 54% of serous carcinomas, 45% of clear cell carcinomas, and 11% of carcinosarcomas. Tumors showing the strongest immunoreactivity for ER and PR were endometrioid adenocarcinomas of all FIGO grades. Occasional clear cell carcinomas and serous carcinomas showed strong PR staining. When they showed any expression at all, ER expression was weak or at most moderate in clear cell carcinoma, serous carcinoma, and carcinosarcoma.

Table 2 Immunohistochemical stains by histologic subtype

	p16 (+)	*ER (+)*	*PR (+)*	*mCEA (+)*	*Vimentin (+)*
FIGO grades 1 and 2	7% (3/42)	84% (31/37)	83% (35/42)	7% (3/42)	90% (38/42)
FIGO grade 3	25% (10/40)	50% (20/40)	42% (16/38)	2.5% (1/40)	81% (30/37)
Serous	92% (22/24)	54% (13/24)	54% (13/24)	13% (3/24)	83% (19/23)
Clear cell	45% (5/11)	9% (1/11)	45% (5/11)	18% (2/11)	91% (10/11)
Carcinosarcoma	67% (6/9)	22% (2/9)	11% (1/9)	0% (0/9)	100% (9/9)
P-value*	<0.001	<0.001	<0.001	0.28	0.49

ER, estrogen receptor; PR, progesterone receptor; mCEA, monoclonal carcinoembryonic antigen; FIGO, International Federation of Gynecology and Obstetrics.
*Significant *P*-values indicate differences between histologic subtypes with respect to immunophenotype.

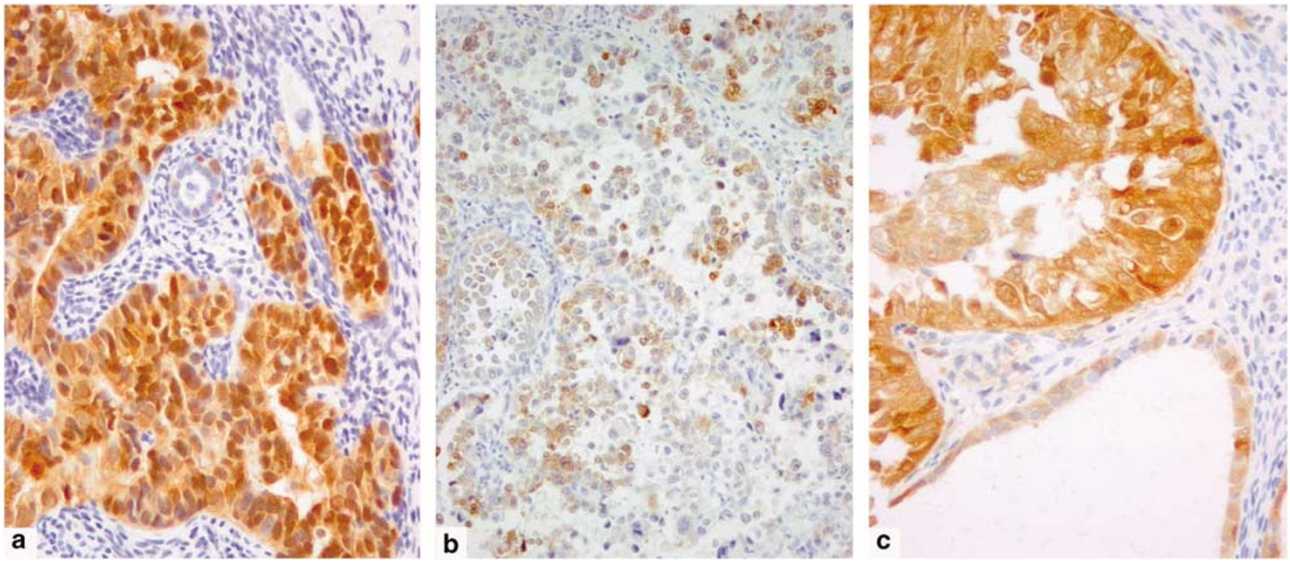

Figure 1 p16 expression in endometrial cancer. (**a**) Diffuse, strong, nuclear, and cytoplasmic labeling of serous carcinoma. Note p16-negative atrophic endometrium (upper right), partly colonized by serous carcinoma. (**b**) A clear cell carcinoma with patchy p16 expression. (**c**) Another serous carcinoma (upper left) juxtaposed with a gland lined by tubal metaplastic cells that focally express p16.

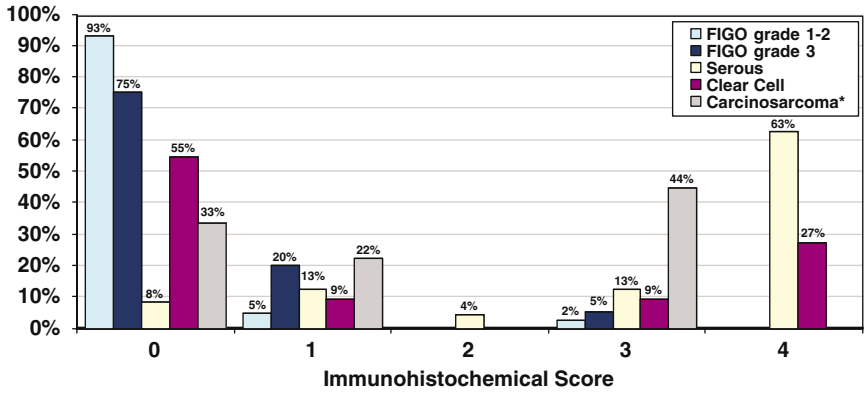

Figure 2 p16 expression in endometrial cancer. FIGO, International Federation of Gynecology and Obstetrics. *Epithelial components only.

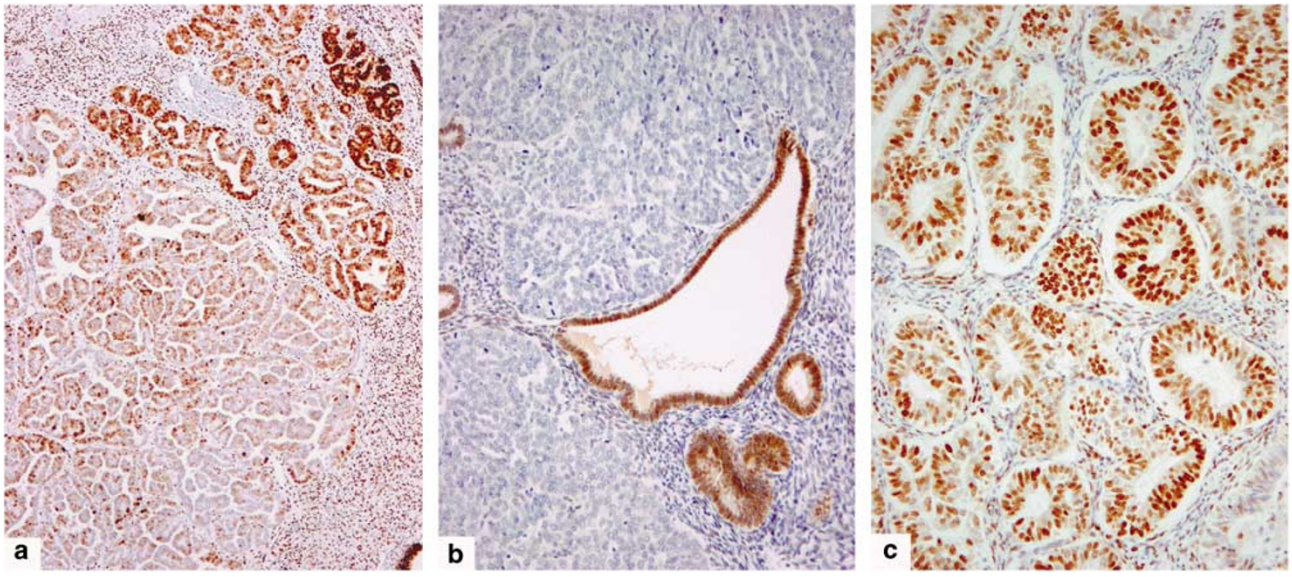

Figure 3 ER expression in endometrial cancer. (**a**) A serous carcinoma with focal ER expression. FIGO grade 3 endometrioid adenocarcinoma (**b**) is negative for ER (note contrasting ER-positive non-neoplastic elements), while (**c**) demonstrates a FIGO grade 1 endometrioid adenocarcinoma with diffuse ER expression.

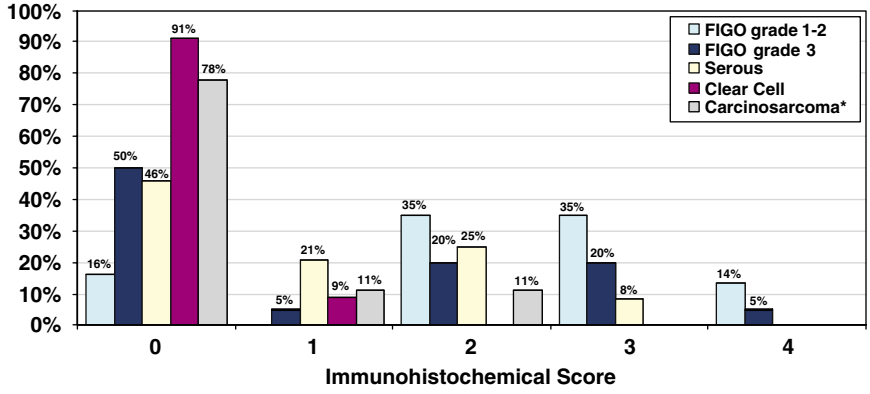

Figure 4 ER expression in endometrial cancer. FIGO, International Federation of Gynecology and Obstetrics. *Epithelial components only.

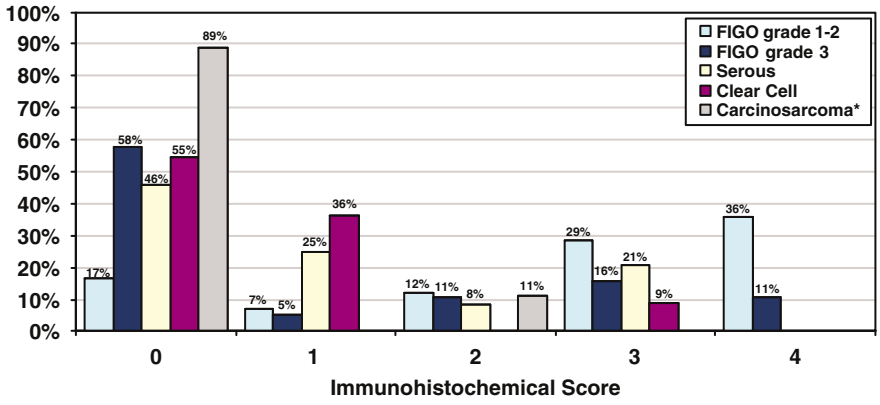

Figure 5 PR expression in endometrial cancer. FIGO, International Federation of Gynecology and Obstetrics. *Epithelial components only.

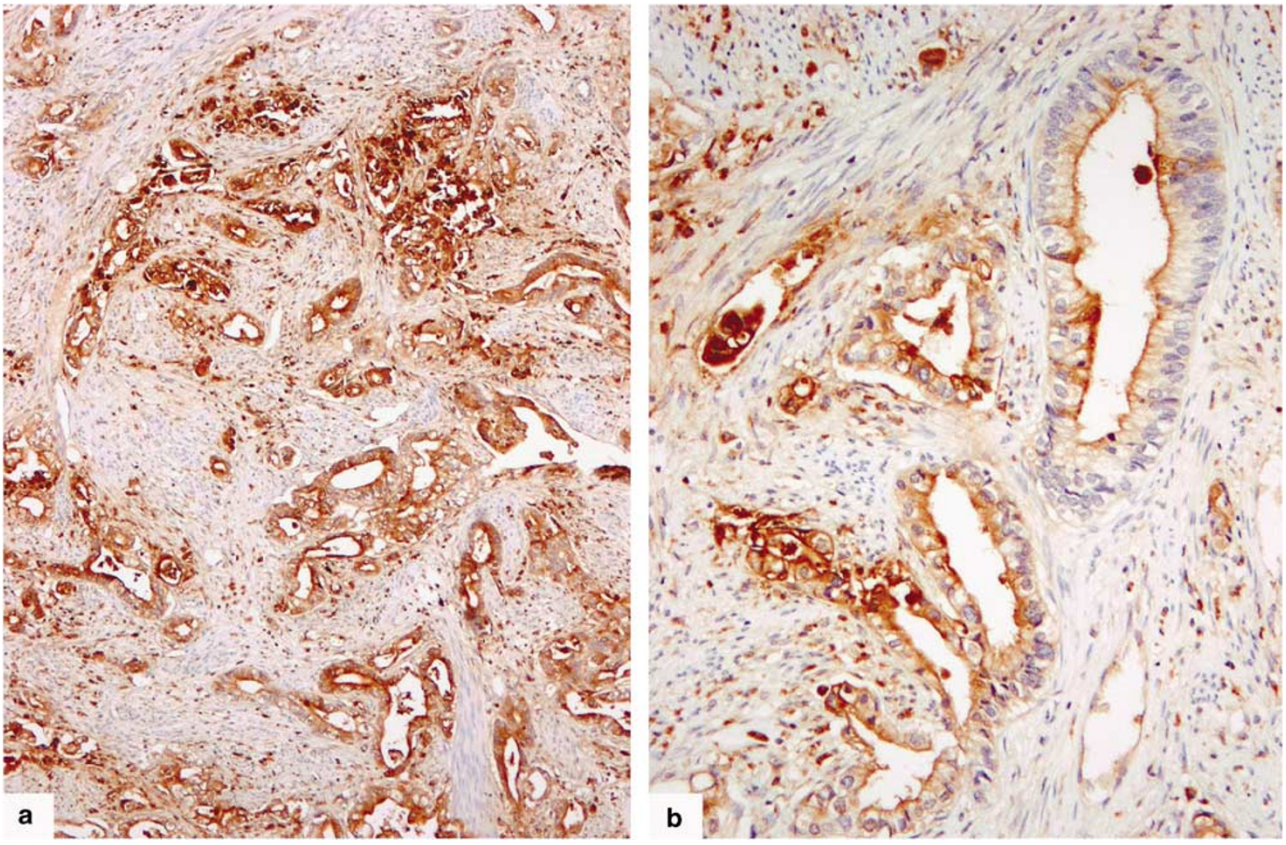

Figure 6 mCEA expression in endometrial cancer. Only rare endometrial adenocarcinomas showed diffuse cytoplasmic mCEA labeling (**a**, an endometrioid adenocarcinoma), which is reported to be relatively specific for endocervical adenocarcinoma. (**b**, another endometrioid adenocarcinoma) shows a more commonly encountered CEA labeling pattern in endometrial cancer, apical accentuation.

Monoclonal CEA (mCEA) (Figures 6 and 7): Most carcinomas were negative for mCEA. Small numbers of endometrioid adenocarcinomas, particularly those with mucinous differentiation, were focally CEA positive. The highest expression rates were seen in serous carcinomas and clear cell carcinomas (13 and 18%, respectively); only rare cases were strongly immunoreactive. CEA immunoreactivity in endometrioid adenocarcinomas showed preferential staining of luminal cell membranes, but occasional cases also showed diffuse cytoplasmic coloration.

Vimentin (Figures 8 and 9): Vimentin was expressed in nearly every tumor, with rates ranging from 81% in FIGO grade 3 endometrioid carcinomas to 100% in carcinosarcomas. Despite the near-uniform rates of positivity for vimentin, there was a broad range of expression strength among tumors. Weakly positive tumors were uncommon, but scores

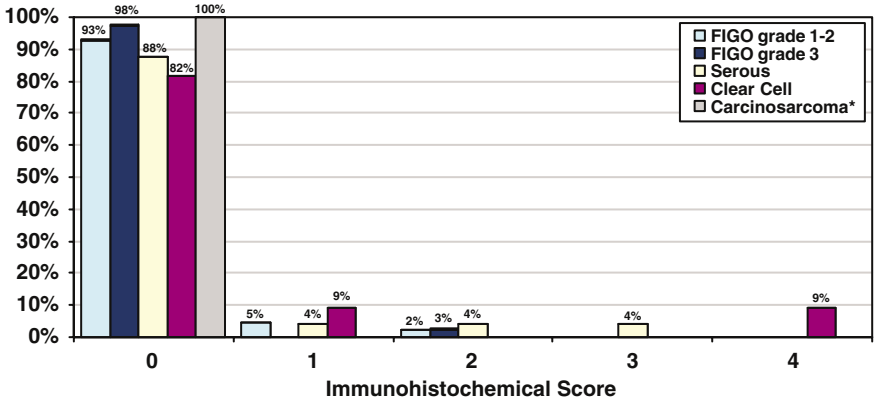

Figure 7 Monoclonal carcinoembryonic antigen (mCEA) expression in endometrial cancer. FIGO, International Federation of Gynecology and Obstetrics. *Epithelial components only.

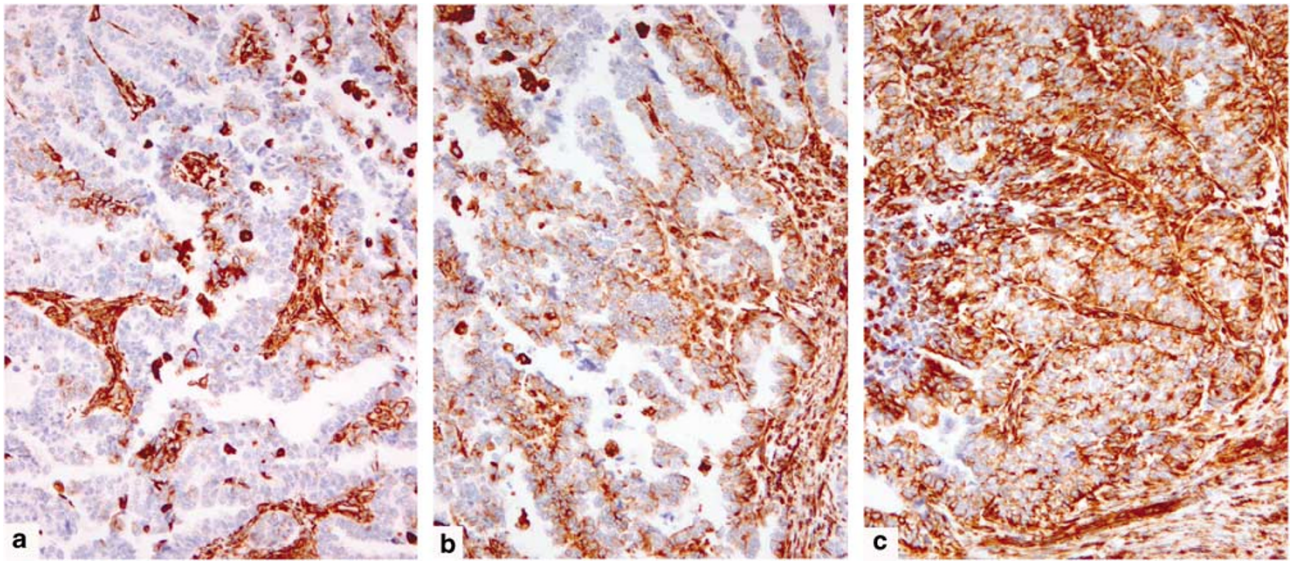

Figure 8 Vimentin expression in endometrial cancer. Rare endometrial cancers are negative for vimentin (**a**, a serous carcinoma) but the vast majority express vimentin either focally (**b**, serous carcinoma) or diffusely (**c**, an endometrioid adenocarcinoma).

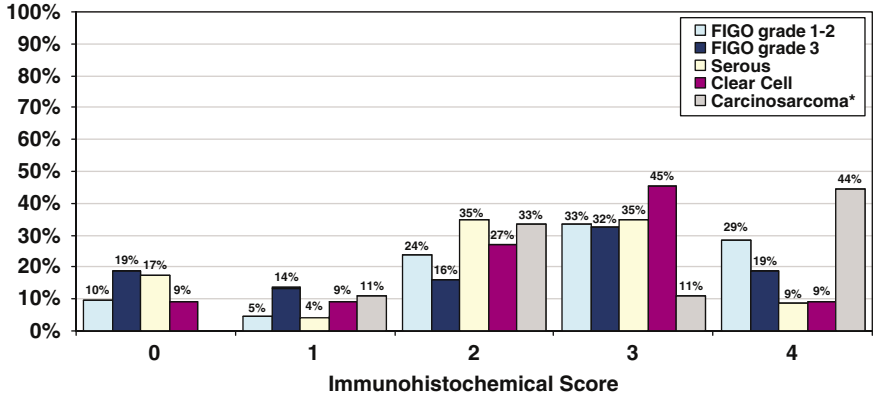

Figure 9 Vimentin expression in endometrial cancer. FIGO, International Federation of Gynecology and Obstetrics. *Epithelial components only.

Table 3 FIGO grades 1 and 2 endometrioid carcinoma immunophenotypes

p16	*ER*	*PR*	*mCEA*	*Vimentin*	*% with this panel*
−	+	+	−	+	70
−	−	−	−	+	8
+	+	+	−	+	5
−	−	+	−	+	2.7
−	−	+	+	−	2.7
−	+	−	−	+	2.7
−	+	+	−	−	2.7
−	+	+	+	+	2.7
+	−	−	−	−	2.7

FIGO, International Federation of Gynecology and Obstetrics; ER, estrogen receptor; PR, progesterone receptor; mCEA, monoclonal carcinoembryonic antigen.

Table 4 FIGO grade 3 endometrioid carcinoma immunophenotypes

p16	*ER*	*PR*	*mCEA*	*Vimentin*	*% with this panel*
−	+	+	−	+	26
−	−	−	−	+	20
−	−	−	−	−	11
+	−	−	−	+	11
−	−	+	−	+	9
+	+	−	−	−	9
−	+	−	−	−	6
−	+	+	−	−	2.9
−	+	+	+	+	2.9
+	+	+	−	+	2.9

ER, estrogen receptor; PR, progesterone receptor; mCEA, monoclonal carcinoembryonic antigen.

Table 5 Serous carcinoma immunophenotypes

p16	*ER*	*PR*	*mCEA*	*vimentin*	*% with this panel*
+	−	+	−	+	22
+	+	+	−	+	17
+	−	−	−	+	13
+	+	−	−	+	13
−	−	−	−	+	9
+	+	−	−	−	9
+	+	+	−	−	9
+	−	−	+	+	4.4
+	+	+	+	+	4.4

ER, estrogen receptor; PR, progesterone receptor; mCEA, monoclonal carcinoembryonic antigen.

of 2–4 were seen in large numbers among every tumor type.

A summary of the most common immunophenotype for each endometrial carcinoma subtype is presented in Tables 3–7. The most common immunophenotype for FIGO grades 1 and 2 endometrioid carcinoma was p16 (−), ER (+), PR (+), CEA (−), and vimentin (+). This phenotype was seen in 70% of FIGO grades 1 and 2 endometrioid carcinomas. This was also the most common immunophenotype encountered in FIGO grade 3 endometrioid carcinomas, but only 26% of such tumors demonstrated this phenotype. A sizable proportion of tumors in the FIGO grade 3 category lacked ER and PR expression and many expressed p16. In all, 22% percent of serous carcinomas were p16 (+), ER (−), PR (+), CEA (−), and vimentin (+)—the most common immunophenotype among serous carcinomas. Occasional serous carcinomas also showed ER expression. The most common clear cell carcinoma immunophenotype, seen in 27% of such tumors, was p16 (+), ER (−), PR (−), CEA (−), and vimentin (+). Many clear cell carcinomas, however, lacked p16 expression. Clear cell carcinoma was the only endometrial cancer tumor type in which CEA expression appeared in a top-three immunophenotype. The majority of carcinosarcomas (56%) were p16 (+), ER (−), PR (−), CEA (−), and vimentin (+).

Table 6 Clear cell carcinoma immunophenotypes

p16	*ER*	*PR*	*mCEA*	*Vimentin*	*% with this panel*
+	−	−	−	+	27
−	−	−	−	+	18
−	−	+	+	+	18
−	−	−	−	−	9
−	−	+	−	+	9
+	−	+	−	+	9
+	+	+	−	+	9

ER, estrogen receptor; PR, progesterone receptor; mCEA, monoclonal carcinoembryonic antigen.

Table 7 Carcinosarcoma[a] immunophenotypes

P16	*ER*	*PR*	*mCEA*	*vimentin*	*% with this panel*
+	−	−	−	+	56
−	−	−	−	+	22
−	+	+	−	+	11
+	+	−	−	+	11

ER, estrogen receptor; PR, progesterone receptor; mCEA, monoclonal carcinoembryonic antigen.
[a]Epithelial components only.

Discussion

Our results demonstrate that the immunophenotypes of endometrial cancers are diverse, indicating that using immunohistochemistry to define the site of origin of a tumor in a curettage specimen can be complicated and subject to error. We also encountered wide variations in distribution and intensity of expression with each marker. p16 expression was common in the epithelial component of endometrial carcinosarcomas and endometrial clear cell carcinomas and was ubiquitous in serous carcinomas where the expression was typically strong and diffuse. Although ER and PR were expressed in many endometrioid adenocarcinomas, a significant proportion of these tumors were negative. Our data also confirmed that CEA is only rarely expressed in any endometrial carcinoma and that vimentin

expression is typical of endometrial carcinomas regardless of histologic subtype; however, there were large numbers of endometrial cancers that expressed vimentin only focally or in a patchy distribution.

p16 is a protein encoded by *p16INK4a.* It normally blocks progression of the cell cycle by inhibiting CDK complex formation with the retinoblastoma protein (RB).[12–14] It may be inactivated by mutation or by promoter hypermethylation. In gynecologic specimens, p16 immunohistochemistry has been used as an indirect assay for HPV infection[1,15,16] and an even more indirect method of determining the primary site of origin[1–3] (in human papillomavirus (HPV)-associated cervical cancers, viral oncoproteins E6 and E7 bind activated RB with consequent upregulation of p16 and promotion of DNA synthesis[15,17]). Well-recognized problems with this approach have been published; p16 expression has been described in non-neoplastic ciliated cells, the cells of tuboendometrioid metaplasia[16,18,19] and even in endometrial cancer.[1,2,20] In endometrial cancer, the expression pattern is generally described as weak and patchy, in contrast to the strong immunoreaction typically encountered in endocervical adenocarcinomas of the usual type.[1,2] The mechanism of p16 expression in metaplastic cells and endometrial cancer has not been determined. We confirmed the generally weak and focal expression pattern in FIGO grades 1 and 2 endometrioid carcinomas, but also noted stronger expression in FIGO grade 3 endometrioid carcinomas, clear cell carcinomas and in the epithelial component of carcinosarcomas. Serous carcinomas showed strong and diffuse expression of p16, suggesting this could be exploited for the differential diagnosis with endometrioid adenocarcinoma. This should be studied in greater detail.

ER and PR expression are known to be common in well-differentiated endometrioid adenocarcinomas of endometrium, although less has been published about ER and PR expression in FIGO grade 3 endometrioid adenocarcinomas,[21] clear cell carcinomas,[22–24] and carcinosarcomas.[25,26] Reports of ER and PR expression in serous carcinoma are not uniform. Serous carcinoma was erroneously considered a largely ER- and PR-positive tumor in the preimmunohistochemistry era. This was due to methods that failed to discriminate between ER/PR-positive stromal elements and carcinoma.[27–29] Following that, serous carcinoma began to be described as a tumor largely negative for ER/PR;[30–32] this highlighted its distinction from most endometrioid carcinomas and conformed to the dualistic endometrial carcinogenesis model of Bokhman.[33] Our data here suggest that ER/PR values for serous carcinoma are intermediate. Admittedly, while overall rates of ER/PR expression in serous carcinoma are generally comparable to that of FIGO grade 3 endometrioid carcinomas, which has been reported previously,[21] the intensity and staining distribution of positive cases was quantitatively and qualitatively less. The epithelial component of carcinosarcoma was only very rarely positive for ER/PR, and ER expression was very uncommon in clear cell carcinoma. If an inappropriate emphasis is placed on immunohistochemistry results, the sufficient numbers of ER/PR-negative cases in each category might cause diagnostic difficulties with histologically similar adenocarcinomas, notably endocervical adenocarcinoma.

CEA has been touted as a good discriminatory marker for endometrial carcinoma *vs* histologic mimics, including endocervical carcinoma,[4–7,34] because endometrial carcinoma is usually CEA-negative and endocervical carcinoma is usually positive. Dallenbach-Hellweg *et al*,[5] however, reported that both endometrial and endocervical mucinous carcinomas expressed CEA; this was refuted by Kamoi *et al*,[9] who reported significantly more CEA expression in endocervical adenocarcinomas of the usual type compared to endometrioid adenocarcinomas of endometrium and endocervix *and* mucinous endometrial adenocarcinomas. The type of antibody used (polyclonal *vs* monoclonal) has some significance. According to Dabbs *et al*,[6] although endometrial adenocarcinomas were largely negative for CEA, the rate of CEA positivity varied in endocervical adenocarcinomas, with significantly higher rates observed with monoclonal CEA as compared to polyclonal CEA. In our study, the only endometrial carcinoma histologic subtypes that expressed CEA in any significant numbers were clear cell and serous carcinomas. Expression in clear cell carcinomas has also been reported by Dallenbach-Hellweg *et al*.[5] With only rare exceptions, positive cases here contained only scattered and weakly immunoreactive cells, most in a membrane distribution. McCluggage *et al*[7] reported membrane expression of mCEA in the glandular component of endometrioid carcinomas of the endometrium that contrasted with the much more common cytoplasmic localization in endocervical adenocarcinomas. Unlike ER, PR, and p16, which showed significant variability in expression between subtypes of endometrial cancers, CEA expression was more uniformly negative, suggesting that it is a better candidate for a site-specific marker.

Vimentin is characteristically positive in endometrial cancer, another point that our study confirms. Of interest is the very heterogeneous distribution and staining patterns seen in many cancers; a number of cases showed only focal and weak vimentin staining. This point calls into question its value as a discriminatory marker for gynecologic carcinomas represented in biopsy or curettage material.

Endometrial cancers demonstrate substantial immunophenotypic diversity that remained apparent even within groups of similar histologic subtype and grade. ER, PR, and p16 expression was more illustrative of tumor type and degree of differentiation than they were of endometrial origin.

In contrast, the vimentin-positive/CEA-negative phenotype remained the most constant among all endometrial cancers. These data underscore some of the problems that result from over-reliance on immunohistochemistry when differentiating between endometrial and endocervical adenocarcinomas, particularly. Traditional methods, including detailed morphologic study and clinical and radiologic correlation, should not be discounted.

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