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Original Article

Endometrioid Squamous Proliferations of the Endometrium Express Alpha-Methylacyl-CoA Racemase (P504s)

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Summary: Squamous morular metaplasia is closely associated with endometrioid proliferative lesions, such as endometrial intraepithelial neoplasia, whereas endometrioid adenocarcinomas (endometrioid carcinoma) may also demonstrate squamous differentiation (morular or nonmorular). Alpha-methylacyl-CoA racemase (AMACR; P504s) is an immunohistochemistry marker expressed in many tumors, including prostate adenocarcinoma, renal cell carcinoma, and a subset of gynecologic tumors, predominantly of clear cell histology. In small biopsy samples, the distinction between cervical high-grade squamous intraepithelial lesions (HSILs) involving endocervical glands from endometrioid squamous proliferation can be challenging, given the anatomic vicinity and some degree of morphologic overlap. Following the observation of AMACR positivity by immunohistochemistry within squamous morules in an index case, 35 endometrial samples containing squamous morular metaplasia (25) and nonmorular squamous metaplasia (10), and 32 cases of cervical HSIL involving endocervical glands were stained with AMACR. The endometrial cohort consisted of 2 benign anovulatory endometrium, 7 endometrial polyps, 7 endometrial intraepithelial neoplasia, 4 atypical polypoid adenomyomas, and 15 endometrioid adenocarcinomas (endometrioid carcinoma). Positive cases were scored as diffuse ($\geq 50\%$) or focal ($< 50\%$). AMACR staining was present in 96.7% of endometrial squamous lesions, including 14 (93.3%) of endometrioid carcinomas, and in all cases of endometrial intraepithelial neoplasia, endometrial polyps, atypical polypoid adenomyomas, and anovulatory endometrium with squamous morular metaplasia or nonmorular squamous metaplasia. In comparison, only 2 cases (5.8%) of cervical HSIL demonstrated positivity with AMACR. In conclusion, AMACR can reliably differentiate the cervical versus endometrial origin of squamous lesions in small biopsy specimens. **Key Words:** Endometrial carcinoma—Hyperplasia—Morular metaplasia—Squamous—AMACR—Immunohistochemistry.

Metaplastic changes of the endometrium are relatively common, (1–5) and include squamous, mucinous, tubal, eosinophilic, and hobnail, among others. Squamous morular metaplasia (SMM) is a distinct type of endometrial metaplasia closely associated with endometrioid proliferative lesions, such as endometrial intraepithelial neoplasia (EIN), (6) whereas endometrial endometrioid carcinoma (EMCA) (7) may also demonstrate squamous differentiation (morular or nonmorular).

It is estimated that about 13% to 25% of EMCAs exhibit some degree of squamous differentiation, most commonly low-grade tumors. (8)

The significance of SMM is dependent on the associated diagnosis; however, it differs from most other types of endometrial metaplasias as it carries a

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mildly increased risk of subsequent endometrial cancer, in special if associated with glandular crowding. The risk of finding endometrioid carcinoma on follow-up sampling in cases of isolated SMM is reported as <5%, whereas in cases of SMM associated with gland crowding the risk is higher (14%). (9,10)

Alpha-methylacyl-CoA racemase (AMACR; p504s) is an enzymatic protein with a role in branched-chain fatty acid metabolism in human cells.(11) AMACR immunohistochemistry (IHC) expression was initially identified in neoplastic prostatic tissue, (12) but further studies have reported its expression in many other solid tumors, (13–16) including endometrial clear cell carcinoma (CCC) (14,15) and CCCs of the urogenital tract. (16–18) Less commonly, Mullerian adenocarcinomas of non-clear cell histology may also be focally positive for AMACR, including 15% of endometrial serous carcinomas, 22% of endometrial endometrioid carcinomas, and in up to 11.8% of other ovarian carcinomas. (19)

The goal of this study was to evaluate the expression of AMACR IHC in squamous lesions of the endometrium, following the observation of diffuse positivity for this marker within the squamous component of an index case of EMCA. Cases of cervical high-grade intraepithelial lesions (HSIL) were also analyzed as some morphologic overlap may exist between SMM and HSIL involving endocervical glands.

MATERIALS AND METHODS

This retrospective study was conducted after Institutional Review Board (IRB) approval, using in-house patient samples retrieved from the surgical pathology database of the University of Miami and Jackson Health System. A total of 35 endometrial samples (21 biopsies, 7 curettages, and 7 resection specimens) containing SMM (25) and nonmorular squamous metaplasia (NMSM; 10), and 32 cases of HSIL involving endocervical glands were identified, with total samples of 67 in a 5-year period (2016–2021). Representative hematoxylin and eosin-stained slides were reviewed by 2 pathologists to confirm the histologic diagnoses and select the blocks for IHC. AMACR staining was performed using clone IR606 Agilent "ready to use," with preconditions in Leica ER2 for 20 minutes at 100°C. Negative and positive controls were appropriately obtained. Prostate tissue was used as a positive control.

Results were classified as positive or negative if AMACR cytoplasmic staining was present in ≥1% or <1% of squamous areas, respectively. Positive cases were subclassified as focal (< 50%) or diffuse (> 50%).

RESULTS

The endometrial cohort consisted of 2 benign anovulatory endometrium, 7 endometrial polyps (EMPs), 7 EIN, 4 atypical polypoid adenomyoma (APA), and 15 EMCA (all low grade; 13 FIGO Grade 1 and 2 FIGO Grade 2), all showing squamous foci. The 20 noncancerous samples demonstrated SMM, whereas 10 of the EMCA cases had NSMM and 5 had SMM.

AMACR IHC was positive in 96.7% of endometrial squamous lesions, including EMCA (14/15), EIN (7/7), EMP (7/7), APAs (4/4), and anovulatory endometrium cases (2/2). Most positive cases (25/35; 71%) showed diffuse labeling; it was focal in 9/35 cases (26%); 1 case was negative.

The diffuse cases comprised 1/2 of anovulatory endometrium, 5/7 EMP, 5/7 EIN, 4/4 APA, and 6/6 EMCA with SMM, whereas it was focal in the remainder. Among the EMCA cases with NMSM, 4/9 showed focal positivity. The only case negative for AMACR corresponded to EMCA with NMSM.

Only 2 cases (2/32; 5.8%) of endocervical glands involved by HSIL demonstrated positivity with AMACR, both showing diffuse expression.

Nonspecific focal staining in the glandular (non-squamous) tissue was observed in 14/35 endometrial cases (including in the nonlesional tissue), while no cervical samples demonstrated AMACR positivity. Tables 1 and 2 summarizes these findings.

TABLE 1. *Percentage of positive AMACR expression in HSIL involving endocervical glands and endometrial squamous lesions*

		Endometrial squamous lesions			
IHC	HSIL; n (%)	EMCA; n (%)	EIN; n (%)	EMP/NE; n (%)	APA; n (%)
AMACR	2/32 (5.8)	14/15 (93)	7/7 (100)	9/9 (100)	4/4 (100)

AMACR indicates Alpha-Methylacyl-CoA Racemase; APA, atypical polypoid adenomyoma; EIN, endometrial intraepithelial neoplasia; EMCA, endometrioid carcinoma; EMP, endometrial polyp; HSIL, high-grade squamous intraepithelial lesion; IHC, immunohistochemistry; NE, Normal endometrium.

TABLE 2. *Endometrial squamous lesions exhibiting SMM and NMSM with diffuse versus focal AMACR expression*

Diagnosis	SMM vs NMSM	Diffuse	Focal	Negative
Benign anovulatory endometrium	SMM (2)	1	1	0
EMP	SMM (7)	5	2	0
EIN	SMM (6)	5	2	0
	Mixed SMM and NMSM (1)			
APA	SMM (4)	4	0	0
EMCA	SMM (6)	6	—	—
	NMSM (9)	4	4	1

AMACR indicates alpha-methylacyl-CoA racemase; APA, Atypical polypoid adenomyoma; EIN, endometrial intraepithelial neoplasia; EMCA, endometrioid carcinoma; EMP, endometrial poly; NMSM, nonmorular squamous metaplasia; SMM, squamous morular metaplasia.

DISCUSSION

The replacement of endometrial glandular epithelium with the cell types not generally seen in endometrial glands is referred to as endometrial metaplasia, best characterized by Hendrickson and Kempson in 1980. (20) Endometrial metaplasia can be associated with hyperestrogenism, inflammation, repeated irritation, EMP, or neoplastic process (endometrial hyperplasia or carcinoma). (21) Although some epithelial metaplasias (eg, squamous or mucinous) may coexist with endometrial hyperplasia or carcinoma, this is generally not true for other types, such as tubal, eosinophilic, or stromal metaplasias. (22–24)

Most SMMs lack estrogen receptor and p63 expression but tend to be positive for CDX-2, CD10, and beta-catenin. (25–27) This immunoprofile is in contrast to typical squamous elements, which are usually positive for CD10 and p63. (28) More recently, SATB2 was described as a positive marker in SMM, (29) and based on the results from our study, AMACR is another IHC consistently expressed in SMM and NMSM.

SMMs are frequently present in uterine endometrioid adenocarcinoma and can mimic areas of solid tumor growth. When accounting for the diagnosis and grading of malignancy, areas of SMM (or NMSM) must be "extracted" from the underlying glands to avoid tumor overdiagnosis and upgrade, especially when dealing with scant biopsy material. (30)

Another diagnostic pitfall in certain endometrial samples is that detached fragments of the high-grade squamous intraepithelial lesion (HSIL) or squamous cell carcinoma can occasionally be present either in endometrial sampling procedures that capture cervical tissue or as a biological extension of the cervical pathology in the endometrium. Although there are some morphologic features that can help distinguish HSIL/squamous cell carcinoma from SMM/NMSM, such as associated nuclear hyperchromasia, enlargement, pleomorphism, and mitotic activity (findings usually absent SMM/NSMM), there are instances in which definitive classification may be difficult.

Immunostain for p16, a well-known surrogate marker for high-risk human papillomavirus genetic material in carcinomas and premalignant lesions, can be positive in SMM despite the lack of association with human papillomavirus, precluding its utility in this differential. Blanco et al. (25) reported p16 expression in squamous differentiation (111/136, 82%), morular metaplasia (22/25, 88%), and endometrioid adenocarcinoma (EMCA) (154/163, 94%). However, p16 staining interpretation is context-dependent and overexpression (including diffuse immunoreactivity) can be seen in other non-human papillomavirus-related lesions of the female genital tract, such as high-grade tubo-ovarian serous carcinoma, uterine serous carcinoma, and leiomyosarcoma, among other entities.

Fadare et al. (19) assessed the utility of AMACR IHC in distinguishing endometrial CCC from serous carcinoma and EMCA and reported that AMACR expression was observed in CCC (75%), serous carcinoma (15%), and EMCA (22%). Hence, it is important to point out that not only cases of carcinomas of non–clear cell histology may express AMACR IHC, but based on the results from our study, EMCA with squamous differentiation will demonstrate positivity for this marker in most cases. The results of our study are in reconciliation with a recently published study by Arciuolo et al., (31) which concluded that endometrial metaplasia mimicking CCC consistently expresses AMACR, which may be accompanied by HNF1β expression; while Napsin A is consistently negative.

Regarding its clinical significance, for other tumor types, AMACR is reported to be a negative prognostic factor in gastric, colorectal, gallbladder, ovarian, urothelial, renal, and prostate malignancies. (19) In contrast, it is presumed to be a positive prognostic factor in small cell carcinomas of the lung. (32) From a therapeutic perspective, targeted attenuation of AMACR expression has been shown to diminish the growth of prostate cancer cell lines, (33) whereas in endometrioid proliferations it is still of unclear significance for prognostic and/or therapeutic purposes.

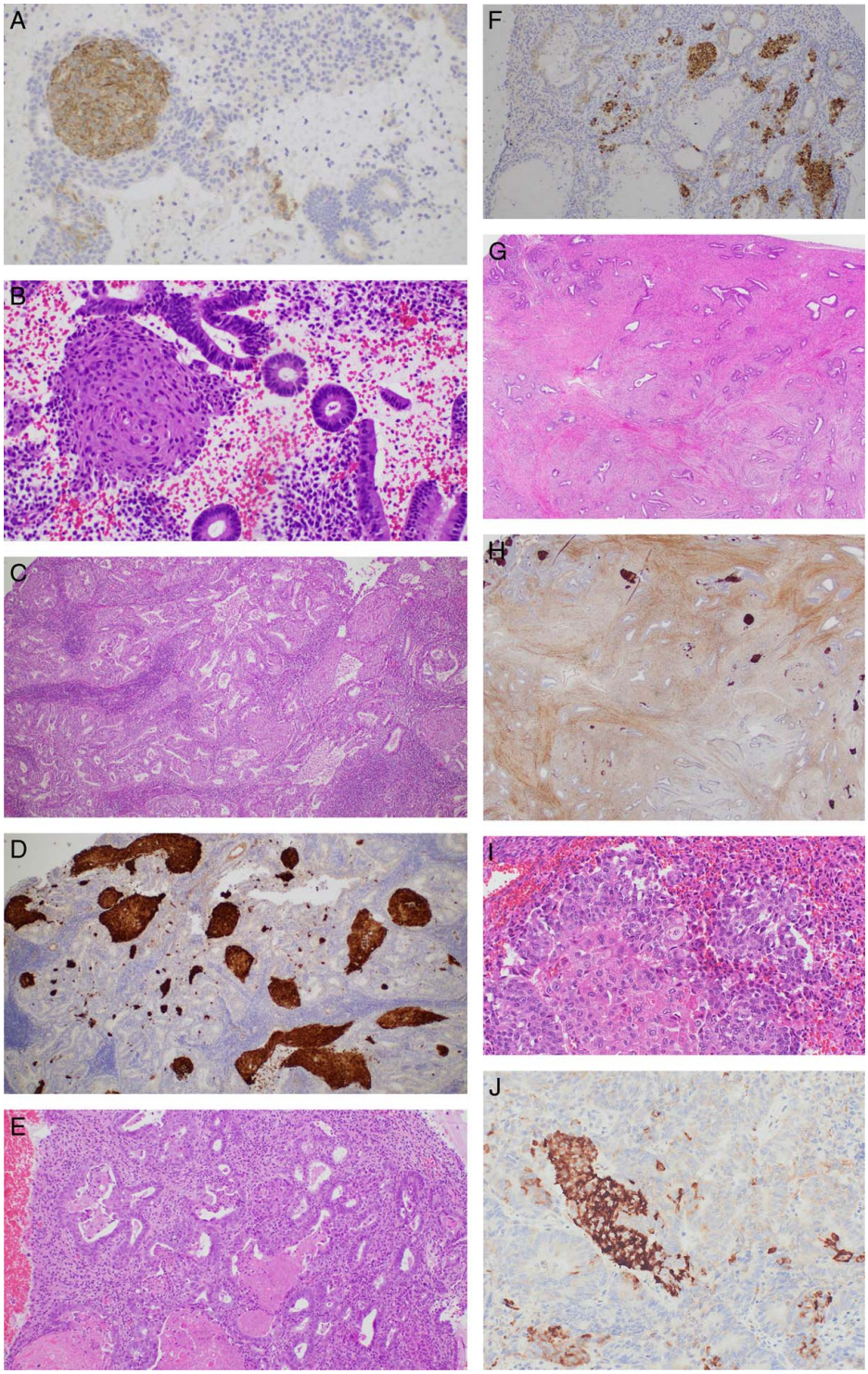

FIG. 1. Examples of endometrial cases demonstrating SMM, NMSM, and corresponding IHC for AMACR. (A and B) Benign anovulatory endometrium (200×) with SMM. (C and D) Endometrial EMCA with both SMM and NMSM (40×). (E, F) EIN with SMM (100×). (G and H) APA (20×) with SMM. (I and J) EMCA with NMSM (200×). AMACR indicates alpha-methylacyl-CoA racemase; APA, atypical polypoid adenomyoma; EMCA, endometrioid carcinoma; EIN, endometrial intraepithelial neoplasia; IHC, immunohistochemistry; NMSM, nonmorular squamous metaplasia; SMM, squamous morular metaplasia.

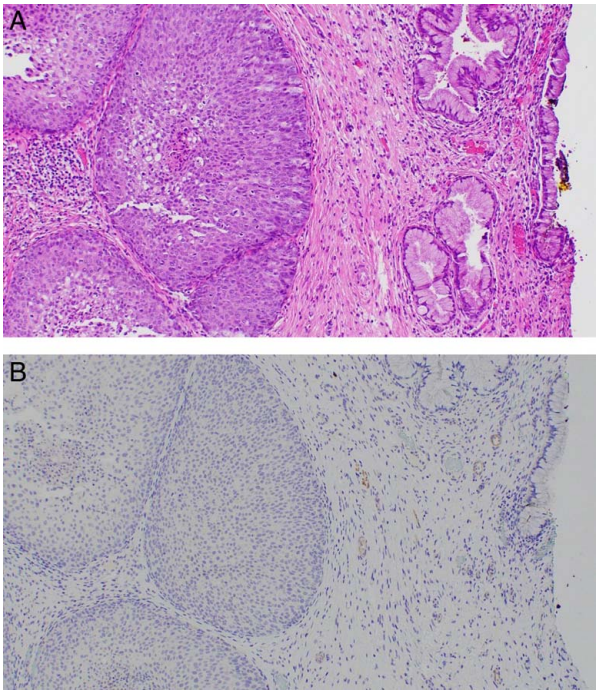

FIG. 2. (A) HSIL involving endocervical glands. (B) IHC for AMACR is negative (both H&E and IHC images were taken at magnification: 100×). AMACR indicates alpha-methylacyl-CoA racemase; H&E, hematoxylin and eosin; HSIL, high-grade squamous intraepithelial lesion; IHC, immunohistochemistry.

CONCLUSION

Our study showed that AMACR IHC expression is significantly higher in endometrial squamous lesions compared with HSIL. Although most endometrial squamous lesions are readily diagnosed based on their distinctive morphologic features, a subset may pose diagnostic difficulties because of morphologic overlap with cervical lesions. AMACR may have a role in diagnostic utility in properly identifying endometrioid proliferative squamous lesions when the diagnostic material is limited. In addition, awareness of this immunohistochemical phenomenon is important to correctly classify cases in which the differential diagnosis is with endometrial clear cell carcinoma (Figs. 1 and 2).

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