

Immunophenotype of Atypical Polypoid Adenomyoma of the Uterus: Diagnostic Value and Insight on Pathogenesis

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Abstract: Atypical polypoid adenomyoma (APA) is a rare uterine lesion constituted by atypical endometrioid glands, squamous morules, and myofibromatous stroma. We aimed to assess the immunophenotype of the 3 components of APA, with regard to its pathogenesis and its differential diagnosis. A systematic review was performed by searching electronic databases from their inception to January 2019 for immunohistochemical studies of APA. Thirteen studies with 145 APA cases were included. APA glands appeared analogous to atypical endometrial hyperplasia (endometrioid cytokeratins pattern, Ki67 \leq 50%, common PTEN loss, and occasional mismatch repair deficiency); the prominent expression of hormone receptors and nuclear β -catenin suggest that APA may be a precursor of “copy number-low,” *CTNNB1*-mutant endometrial cancers. Morules appeared as a peculiar type of hyperdifferentiation (low Ki67, nuclear β -catenin+, CD10+, CDX2+, SATB2+, p63-, and p40-), analogous to morular metaplasia in other lesions and distinguishable immunohistochemically from both conventional squamous metaplasia and solid cancer growth. Stroma immunophenotype (low Ki67, α -smooth-muscle-actin+, h-caldesmon-, CD10-, or weak and patchy) suggested a derivation from a metaplasia of normal endometrial stroma. It was similar to that of nonatypical adenomyoma, and different from adenocarcinoma (Ki67 increase and CD10+ in periglandular stroma) and myoinvasive endometrioid carcinoma (h-caldesmon+ in myometrium and periglandular fringe-like CD10 pattern).

Key Words: adenomyofibroma, premalignant, immunohistochemistry, mullerian tumor

(*Appl Immunohistochem Mol Morphol* 2019;00:000–000)

Atypical polypoid adenomyoma (APA) is an uncommon uterine lesion characterized by a proliferation of atypical endometrial glands, with squamous morular metaplasia and a typical fibromyomatous stroma.^{1–5}

Received for publication February 16, 2019; accepted April 21, 2019.

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The authors declare no conflict of interest.

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Histologically, APA may be difficult to differentiate from myoinvasive endometrioid adenocarcinoma;¹ furthermore, morular metaplasia may mimic a solid growth pattern.⁶ Such differential diagnosis appears even more important, as APA affects premenopausal and nulliparous women in most cases.^{3–5}

However, despite being regarded as a benign lesion, APA shows significant rates of progression to endometrial cancer, and of recurrence when conservatively treated; these findings, together with the presence of cytologic atypia, support the precancerous nature of APA.^{7,8} The relation of APA with atypical endometrial hyperplasia (AEH, the precursor of endometrioid adenocarcinoma)^{9,10} is still undefined, as well as the origin of the features that distinguish APA from AEH (ie, delimited polypoid appearance, squamous morules, and myofibromatous stroma).¹¹ Furthermore, it is also unclear how APA is related to the 4 molecular categories of endometrial cancer identified by The Cancer Genome Atlas (TCGA), that is, “hypermuted,” “ultramuted,” “copy number-low,” and “copy number-high.”¹²

Although molecular studies of APA have been exceptional,^{11,13,14} most of the scientific evidence with regard to the pathogenesis of APA may be gathered from immunohistochemical studies.^{6,11,13–25} Immunohistochemistry has also played a major role in improving the differential diagnosis of APA.^{6,22,23}

The objective of our study was to provide a complete overview of the immunophenotype of the 3 components of APA (glands, morules, and stroma), to explore old and new insights on its pathogenesis and its differential diagnosis.

MATERIALS AND METHODS

Study Protocol

This study was conducted following a protocol defined a priori. All review stages, including search strategy, study selection, risk of bias assessment, data extraction, and data analysis, were conducted independently by 2 authors (A.T., A.R.). In case of disagreement, consensus was achieved by discussion among authors. The review was reported according to the PRISMA²⁶ statement.

Search Strategy

MEDLINE, Scopus, EMBASE, Web of Sciences, OVID, Google Scholar, and Cochrane Library were

searched from the inception of each database to January 2019. The following combination of text words was used: (atypical polypoid) AND (adenomyoma OR adenofibroma OR adenomyofibroma). References from relevant articles were checked to identify further studies.

Study Selection

All studies reporting immunohistochemical features of APA were included. Exclusion criteria were as follows: same sample as a study already included, case reports, and reviews. No language restrictions were applied,

Risk of Bias Assessment

The risk of bias was assessed in each study, in relation to 5 domains: (1) Selection (were APA specimens selected consecutively? Were period of enrollment and selection criteria reported?); (2) Diagnosis (were histologic slides reviewed to confirm APA diagnosis? Were histologic features of APA presented?); (3) Methodology (were methods for immunohistochemistry clearly described?); (4) Loss (were all included specimens evaluated immunohistochemically?); and (5) Results (were immunohistochemical results clearly and fully presented?). For each domain, the risk of bias was categorized as “low,” “high,” or “unclear,” depending on whether data were “reported and adequate,” “reported, but not adequate,” “not reported,” respectively.

Data Extraction

Data were extracted from each study without modifications. For each study, the main data extracted were sample size, immunohistochemical markers assessed, intensity of distribution of the expression of each marker, and correlation with genetic findings (when possible). Secondary data extracted were country, study design, period of enrollment, location of APA around the uterus, characteristics of the patients, and clinical behavior of APA (ie, rates of recurrence and progression).

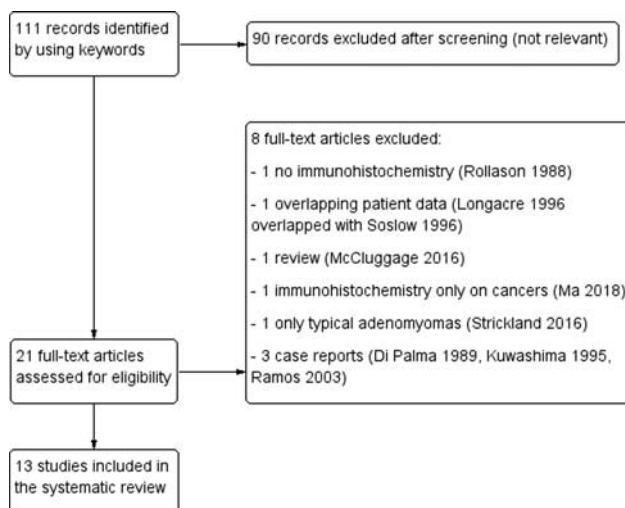


FIGURE 1. Flow diagram of study selection (PRISMA template).

RESULTS

Selection and Characteristics of the Studies

Thirteen studies with a total of 145 APA specimens were included.^{6,11,13,14,16,18,20–25,27} The fully reproducible process of study selection on the electronic database “MEDLINE” is presented in Figure 1. Sampling methods for histologic examination varied among studies, including hysterectomy, polypectomy, curettage, transcervical resection, and hysteroscopic biopsy. Three studies focused in particular on APA morules, comparing them to conventional squamous metaplasia and/or to molar metaplasia in other lesions.^{6,20,21} Four studies also assessed myoinvasive endometrioid adenocarcinoma specimens, to identify diagnostic markers that may allow a differential diagnosis with APA.^{18,22,23,27} In 1 study, APA was compared with adenosarcoma and endometrial polyp.²⁵ Characteristics of the included studies are shown in Table 1.

TABLE 1. Characteristics of the Included Studies

References	Country	Period of Enrollment	Sample Size	Sample Type
Fukunaga et al ¹⁶	Japan	1991-1994	6	Hysterectomy, curettage, polypectomy
Soslow et al ¹⁸	United States	Unclear	23	Curettage, biopsy, hysterectomy, polypectomy
Ota et al ¹³	Spain	Unclear	6	Hysterectomy, polypectomy
Chiarelli et al ²⁰	Italy, Spain	Unclear	4	Curettage, aspiration, hysterectomy
Houghton et al ²¹	United Kingdom	Unclear	3	Unclear
Ohishi et al ²²	Japan	Unclear	7	Curettage, hysterectomy
Horita et al ²³	Japan	2005-2010	6	Curettage, hysterectomy, transcervical resection
Terada ²⁴	Japan	Unclear	5	Hysterectomy, polypectomy
Aggarwal et al ²⁵	United States	Unclear	14	Biopsy, curettage
Takahashi et al ¹¹	Japan	1990-2012	7	Curettage, hysterectomy
Němejcová et al ¹⁴	Czech Republic; United Kingdom	Unclear	21	Hysterectomy, curettage, polypectomy
McCluggage and Van de Vijver ⁶	United Kingdom; Belgium	Unclear	7	Unclear
Lu et al ²⁷	China	2003-2017	36	Polypectomy, hysterectomy

	Selection	Diagnosis	Methodology	Loss	Results
1995 Fukunaga [16]	+	+	+	+	+
1996 Soslow [18]	+	+	+	+	+
2003 Ota [13]	?	+	+	+	+
2006 Chiarelli [20]	?	?	+	?	?
2008 Houghton [21]	+	?	+	+	?
2008 Ohishi [22]	?	+	+	+	+
2011 Horita [23]	+	+	+	+	+
2011 Terada [24]	?	?	+	+	+
2012 Aggarwal [25]	?	?	+	+	-
2013 Takahashi [11]	+	+	+	+	+
2015 Nernejcova [14]	?	+	+	+	+
2018 McCluggage [6]	?	+	+	+	+
2019 Lu [26]	+	+	+	+	+

FIGURE 2. Risk of bias summary: review authors’ judgments about each risk of bias item for each included study. Question mark indicates unclear risk of bias; minus sign, high risk of bias; NA, not applicable; plus sign, low risk of bias.

Risk of Bias Assessment

The risk of bias in the “selection” domain was low in 6 studies (they clearly stated that specimens were consecutive, or reported at least the period of enrollment and inclusion criteria) and unclear in 7 studies.^{6,13,14,20,22,24,25}

For the “diagnosis” domain, the risk of bias was low in 9 studies (histologic slides reviewed, morphologic features of APA well presented) and unclear in 4.^{20,21,24,25}

For the “methodology” domain, the risk of bias was low in all studies (methods for immunohistochemistry clearly described).

For the “loss” domain, 12 studies were considered at low risk (no exclusion of specimens from immunohistochemistry, or exclusion explained by tissue unavailability), and 1 at unclear risk.²⁰

For the “results” domain, 9 studies were at low risk, 2 at unclear risk (APA and other lesions lumped together),^{20,21} and 1 at high risk (discrepancy between text and figures) (Fig. 2).²⁵

Immunohistochemistry

Glands

The glandular component of APA showed positivity for cytokeratins CAM5.2,¹⁸ AE1/AE3, 8, 18, 7, and 19; the expression of cytokeratins 34βE12, 5/6, and 13 was variable; cytokeratins 14 and 20 were negative.²⁴

The expression of the proliferation marker Ki67 was highly variable, with a labeling index (L.I.) ranging from 0 to almost 50%.^{11,23–25,27}

Estrogen and progesterone receptors were expressed strongly and diffusely in 95% to 100% of cases.^{18,24,27}

Among the molecules involved in endometrial carcinogenesis, PTEN was frequently lost (about 1/3 of cases) or deficient, as confirmed by the finding of PTEN mutation on molecular analysis.¹⁴ mTOR was expressed in 90% of cases, with variable intensity.¹⁴ Nuclear expression of β-catenin was observed in 50% to 80% of cases, with high variability in the intensity.^{11,13,14} Among mismatch repair proteins, MLH1 was found to be deficient in only 2 cases (correspondent to MLH1 methylation status), whereas MSH2, MSH6, and PMS2 were always proficient.^{13,14} Expression pattern of p53 was normal, as well as TP53 status (wild type).^{14,23,24}

Among tumor markers, CA125 was always strongly expressed; the expression of CA19.9 was variable, whereas CEA was negative.^{16,24} The expression of p21, cyclin D1 and cyclin E was highly variable.^{11,27}

Expression profile of mucins showed positivity for MUC1 and MUC6 and negativity for MUC2 and MUC5AC.²⁴

Immunohistochemical findings for the glandular component of APA are shown in detail in Table 2.

Morules

Expression profile of cytokeratins showed positivity for cytokeratins 903,²² CAM5.2¹⁸ 8, 18, and 19; weak positivity for cytokeratins 34βE12, 5-6, and 13; and negativity for cytokeratins 7 and 20.²⁰

The expression of estrogen and progesterone receptors was absent, or weaker compared with the glandular component^{18,20,21}; also, cellular proliferation, assessed as Ki67 L.I., was low (0% to 5%).^{11,27}

The expression of the squamous markers p40 and p63 was negative, whereas unexpected expression of CDX2 and SATB2 was observed.^{6,21,27} Unlike the glandular component, and in contrast with conventional squamous metaplasia, morules were positive for CD10.^{11,20–23,25,27}

Nuclear expression of β-catenin was both stronger and more diffuse than in glands.^{11,13,14,20,21,27}

Immunohistochemical findings for the morular component of APA are shown in detail in Table 3.

Stroma

The myofibromatous stroma of APA was always positive for α-smooth-muscle-actin (strong and diffuse positivity in 70% to 100% of cases),^{11,16–18,22–25,27} whereas the expression of desmin, vimentin, CD10, and CD34 was variable;^{11,16,18,22–25} S100 was negative.²⁴

h-caldesmon, a smooth muscle marker, was always completely negative in APA stroma (positive only in vessels).^{23,27} In 1 study, the authors stated in the text that smooth muscle markers (also including h-caldesmon) were positive in the stroma of APA, whereas the figure clearly showed that h-caldesmon was negative.²⁵

TABLE 2. Immunohistochemical Findings in APA Glands

Ki67	
Horita et al ²³	Variable (L.I. 2.9%-44.7%)
Tearada et al ²⁴	Variable (L.I. 3%-12%)
Aggarwal et al ²⁵	More intense than in stroma
Takahashi et al ¹¹	Variable (L.I. 0.8%-30%)
Lu et al ²⁷	Variable (L.I. 20.86 ± 16.51%)
Cytokeratins	
Soslow et al ¹⁸	CAM5.2 positive
Terada ²⁴	CKAE1/AE3, CAM5.2, CK8, and CK18 strongly positive; CK7 and CK19 positive (variable intensity); CK34βE12, CK5/6, and CK13 variable; CK14 and CK20 negative
β-catenin	
Ota et al ¹³	Nuclear in 5/6 cases
Takahashi et al ¹¹	Variably nuclear (L.I. 5%-55.3%)
Nemejcova et al ¹⁴	Nuclear in 12/21 cases
Lu et al ²⁷	Nuclear (focal and weak)
p53	
Horita et al ²³	Always normal
Terada ²⁴	Always normal
Nemejcova et al ¹⁴	Always normal
Estrogen receptor	
Soslow et al ¹⁸	Always prominent
Terada ²⁴	Always diffusely positive
Lu et al ²⁷	Strongly and diffusely positive
Progesterone receptor	
Soslow et al ¹⁸	Always positive (prominent in 22/23 cases)
Terada ²⁴	Always diffusely positive
Lu et al ²⁷	Strongly and diffusely positive
Mismatch repair proteins	
Ota et al ¹³	MLH1 focally negative in 2/6 cases; MSH2 always normal
Nemejcova et al ¹⁴	MLH1, MSH2, MSH6, and PMS2 always normal
Mucins	
Terada ²⁴	MUC1 positive (weak to strong intensity); MUC6 positive (weak intensity); MUC2 and MUC5AC negative
CEA	
Fukunaga et al ¹⁶	Negative
Terada ²⁴	Negative
CA125	
Terada ²⁴	Always positive
CA19.9	
Terada ²⁴	Variable
p21	
Takahashi et al ¹¹	Variable (L.I. 0.7%-18.8%)
Cyclin D1	
Takahashi et al ¹¹	Variably positive (L.I. 17.2%-81.8%)
Lu et al ²⁷	Positive
Cyclin E	
Takahashi et al ¹¹	Variable (null to diffuse)
PTEN	
Nemejcova et al ¹⁴	Null in 6/21 of cases, positive (variable L.I.) in 15/21
GLUT-1	
Nemejcova et al ¹⁴	Always positive (variable intensity)
mTOR	
Nemejcova et al ¹⁴	Positive in 17/19 cases
HNF1β	
Nemejcova et al ¹⁴	Positive in 16/21 cases
EMA	
Terada ²⁴	Negative
SOX9	
Lu et al ²⁷	Positive

APA indicates atypical polypoid adenomyoma; L.I., labeling index.

Ki67 L.I. was sensibly lower than in glands (0% to 10%).^{11,23-25} Estrogen receptor was always expressed, with variable extent and intensity; the expression of progesterone receptor varied.^{18,24}

TABLE 3. Immunohistochemical Findings in APA Morules

CD10	
Chiarelli et al ²⁰	Positive
Houghton et al ²¹	Positive in 16/17 cases
Ohishi et al ²²	Diffuse and strong
Horita et al ²³	Positive
Aggarwal et al ²⁵	Positive
Takahashi et al ¹¹	Positive
Lu et al ²⁷	Positive
β-catenin (nuclear)	
Ota et al ¹³	Positive and stronger than in glands in 4/5 cases
Chiarelli et al ²⁰	Positive
Houghton et al ²¹	Positive in 16/18 cases
Takahashi et al ¹¹	Positive and stronger than in glands
Nemejcova et al ¹⁴	Positive and stronger than in glands in 15/19 cases
Lu et al ²⁷	Positive and stronger than in glands
Estrogen receptor	
Soslow et al ¹⁸	Variable
Chiarelli et al ²⁰	Negative
Houghton et al ²¹	Negative in 12/18 cases
Lu et al ²⁷	Almost null
Progesterone receptor	
Soslow et al ¹⁸	Variable
Chiarelli et al ²⁰	Negative
Lu et al ²⁷	Almost null
Ki67	
Takahashi et al ¹¹	Low (L.I. 0.8%-5%)
Lu et al ²⁷	Low (L.I. 1.52 ± 0.83%)
CDX2	
Houghton et al ²¹	Diffuse in 14/17 cases
Lu et al ²⁷	Diffuse and strong
Cytokeratins (CK)	
Soslow et al ¹⁸	CAM5.2 positive
Chiarelli et al ²⁰	CK8, CK18, and CK19 positive; CK5-6, CK13, and CK34βE12 weak; CK7 and CK20 negative
Ohishi et al ²²	CK903 positive
p63	
Houghton et al ²¹	Negative in 16/17 cases
p40	
Lu et al ²⁷	Null to weak and focal
p21	
Takahashi et al ¹¹	From focal to diffuse
Cyclin D1	
Takahashi et al ¹¹	Always positive (L.I. 35%-76%)
Lu et al ²⁷	Positive (weaker than in glands)
Cyclin E	
Takahashi et al ¹¹	Null to diffuse in all components
GLUT-1	
Nemejcova et al ¹⁴	Always positive (variable intensity)
SATB2	
McCluggage and Van de Vijver ⁶	Diffuse in 38/43 cases
LP34	
Houghton et al ²¹	Diffuse in 17/18 cases
SOX9	
Lu et al ²⁷	Positive (weaker than in glands)

APA indicates atypical polypoid adenomyoma; L.I., labeling index.

Immunohistochemical findings for the stromal component of APA are shown in detail in Table 4.

Immunohistochemical expression of the markers studied in the differential diagnosis between APA and myoinvasive endometrioid adenocarcinoma is detailed in Table 5.

TABLE 4. Immunohistochemical Findings in APA Stroma

αSMA	
Fukunaga et al ¹⁶	Always strong and diffuse
Soslow et al ¹⁸	Always positive (strong and diffuse in 17/23 cases)
Ohishi et al ²²	Always diffuse and strong
Horita et al ²³	Always positive
Terada ²⁴	Always diffusely positive
Aggarwal et al ²⁵	Positive
Takahashi et al ¹¹	Strong and diffuse
Lu et al ²⁷	Strongly positive
CD10	
Ohishi et al ²²	Negative or focal and weak
Horita et al ²³	Negative or partially positive
Terada ²⁴	Always positive
Takahashi et al ¹¹	Negative or focal and weak
Lu et al ²⁷	Focally positive
Desmin	
Fukunaga et al ¹⁶	Positive in 30%-85% cells
Soslow et al ¹⁸	Variable (negative to focal and intense)
Ohishi et al ²²	Negative or weak and focal
Terada ²⁴	Variable
Aggarwal et al ²⁵	Positive
Ki67	
Horita et al ²³	Variable (L.I. 0.3%-10.8%)
Terada ²⁴	Variable (L.I. 1%-8%)
Aggarwal et al ²⁵	Low (L.I. <5%)
Takahashi et al ¹¹	Low (L.I. 0.8%-2.8%)
H-caldesmon	
Horita et al ²³	Negative (positive only in vessels)
Aggarwal et al ²⁵	Unclear (positive in text, negative in figure)
Lu et al ²⁷	Negative
Vimentin	
Fukunaga et al ¹⁶	Always diffuse
Terada ²⁴	Positive in 4/5 cases
Estrogen receptor	
Soslow et al ¹⁸	Always positive
Terada ²⁴	Always diffusely positive
Progesterone receptor	
Soslow et al ¹⁸	Variable
Terada ²⁴	Always diffusely positive
S100	
Terada ²⁴	Negative
HHF35	
Fukunaga et al ¹⁶	Diffuse and intense
CD34	
Soslow et al ¹⁸	Variable (negative/weak in 21/22 cases)
p21	
Takahashi et al ¹¹	Variable (L.I. 0.6%-51.4%)
Cyclin D1	
Takahashi et al ¹¹	Variable (L.I. 1.7%-38.1%)
Cyclin E	
Takahashi et al ¹¹	Null to diffuse
EMA	
Terada ²⁴	Negative
SATB2	
McCluggage and Van de Vijver ⁶	Diffusely positive

APA indicates atypical polypoid adenomyoma; L.I., labeling index.

DISCUSSION

APA Glands, AEH, and TCGA Groups

APA glands are indistinguishable from AEH,¹¹ and the cytokeratins' expression pattern does not differ from

TABLE 5. Immunohistochemical Comparison Between APA and Myoinvasive Endometrioid Adenocarcinoma

	APA	Myoinvasive Endometrioid Adenocarcinoma
Glands		
Cytokeratin CAM5.2 ¹⁸	Always positive	Always positive
Estrogen receptor ¹⁸	Always positive	Positive in 90%
Progesterone receptor ¹⁸	Always positive	Positive in 90%
p53 ²³	Always normal (L.I. 2.1%-40.1%)	Always normal (L.I. 0.2%-14.4%)
Ki67 ²³	Variable (L.I. 2.9%-44.7%)	Variable (L.I. 14.4%-92.3%)
Stroma		
αSMA ^{22,23,27}	Always positive	Always positive
Desmin ^{18,22}	Positive in 17/30 cases	Positive in 26/29 cases
CD34 ¹⁸	Positive in 9/22 cases	Positive in 4/8 cases
CD10 ^{22,23}	Negative or weakly positive	Fringe-like pattern (5%-100% of glands)
h-caldesmon ^{23,27}	Always negative	Always positive
Estrogen receptor ¹⁸	Always positive (variable intensity)	Variable
Progesterone receptor ¹⁸	Variable	Variable
p53 ²³	Always normal (L.I. 1.3%-32.9%)	Always normal (L.I. 0.1%-5.8%)
Ki67 ²³	L.I. 0.3%-10.8%	L.I. 1.5%-7.2%

APA indicates atypical polypoid adenomyoma; L.I., labeling index.

other endometrioid proliferations.^{15,18,24} The proliferation index, evaluated as Ki67 L.I., can be moderately increased in APA, with a significant overlap of values with endometrioid carcinoma. However, whereas Ki67 L.I. seems to never exceed 50% in APA, it could be even over 90% in endometrioid carcinoma.^{23,27}

These findings, together with the possibility of deficient expression of PTEN, support the premalignant nature of APA and its similarity to AEH.^{11,14,23,28-33} In fact, it has been suggested that APA may be a localized form of AEH¹¹; furthermore, the use of IHC for distinguishing APA from AEH is discouraged by ESGO guidelines.³⁴ In this regard, it would be interesting to assess APA for other molecules that are frequently altered in AEH, such as Bcl-2, ARID1A, and PAX2.³⁵⁻³⁷

Remarkably, APA is reported to be associated with mismatch repair deficiency in the 2014 WHO classification of gynecologic tumors.⁹ On the basis of only 1 study,¹³ such a statement seems to suggest a particular association of APA with microsatellite instable endometrial cancers of the TCGA "hypermutated" group.^{12,38} However, according to our review, mismatch repair proteins are only rarely deficient in APA, similarly to AEH.^{13,14,39}

Estrogen and progesterone receptors were always found to be strongly and diffusely expressed in APA glands, whereas AEH may sometimes show low or absent expression.⁴⁰ Consistently, progestins have been used as a conservative treatment for APA.⁴¹⁻⁴³ Interestingly, our

previous study showed that the addition of progestins did not significantly improve the outcomes of APA treatment if compared with hysteroscopic resection alone.⁵ By contrast, the addition of progestins is required for AEH.^{44,45} Reasons for such a difference are unclear, although it might be due to the delimited polypoid morphology of APA, which might facilitate a complete excision.

Strong hormonal receptors' expression was also observed in cancers developed from APA, which usually are low-grade endometrioid carcinomas.⁴⁶ These findings suggest that most APAs may be precursors of endometrial cancers of the "copy number-low" group identified by TCGA.¹² Such hypothesis may also be supported by the characteristic nuclear expression pattern of β -catenin in APA epithelial component (more prominent in morules). In fact, Takahashi et al¹¹ showed that nuclear β -catenin reflected the presence of *CTNNB1* mutations in APA, and *CTNNB1* mutations are particularly frequent in the "copy number-low" group.¹² Consistently, in our previous study, we showed that nuclear expression of β -catenin was an accurate immunohistochemical surrogate of *CTNNB1* exon 3 mutations in endometrial cancer.⁴⁷

Given these observations, the main molecular divergence between APA and AEH in endometrial carcinogenesis may not lie in mismatch repair deficiency, but in *CTNNB1* mutation, which might also be responsible for APA peculiar histology (see below). Indeed, nuclear expression of β -catenin is much less common in AEH.³⁹

Interestingly, *CTNNB1* mutations have a prognostic significance in the "copy number-low" cancers, identifying cases at worse prognosis.⁴⁸ Consequently, *CTNNB1* mutation has been proposed as a marker to define a separate subgroup within the copy number-low group.⁴⁹ It might reasonably be hypothesized that most APAs represent precursors of cancers of this specific subgroup.

As in AEH, the main available marker of the "copy number-high" group, namely overexpression of p53,³⁸ was never observed in APA. Finally, the relation of APA with the "ultramutated" group is still unclear.

APA Morules, Squamous Differentiation, and Solid Tumor Growth

A characteristic feature of APA epithelial component is the presence of squamous morules, which can be observed less commonly in AEH and in endometrioid carcinoma. Despite being referred to as "squamous morular metaplasia," their immunophenotype is completely different from that of conventional squamous metaplasia. In fact, they are usually negative for the squamous markers p40 and p63, and diffusely positive for CD10 and the heterotopic markers CDX2 and SATB2.^{6,20,21,27} In this regard, it has been proposed that morules are not truly squamous and that morular pattern is a separate differentiation, which should be referred to as "morular metaplasia."⁶

On differential diagnosis, such peculiar immunophenotype may also allow differentiating morular metaplasia from a cancer with solid growth.⁶ In addition, the insignificant Ki67 L.I. also would be incompatible with a

solid carcinoma. In fact, in APA, as in AEH, morules seems to be an "inert" component.^{11,27,50} A prodifferentiative mechanism, which involves p21, cyclin D1, and β -catenin (all overexpressed in APA morules), has been suggested as the origin of morular metaplasia.¹¹ In particular, nuclear expression of β -catenin is almost always present in APA morules, appearing stronger and more diffuse than in glands.^{11,13,14,20,21,27}

APA Stroma, Mullerian Tumors, and Myoinvasive Cancer

The origin of the peculiar stroma of APA is unclear. A metaplastic change of the normal endometrial stroma to a myofibromatous stroma has been proposed as the main mechanism.^{4,11,22} The combination of strong and diffuse positivity for α SMA and complete negativity for h-caldesmon appears as a hallmark of the stroma of APA and of a subset of nonatypical adenomyomas.^{23,27,51} Weak and patchy CD10 expression may still be observed, indicating the presence of endometrial stromal cells admixed with the fibromyomatous component.^{11,22,23} Hormonal action might be the driver mechanism for the metaplastic stromal change. Such hypothesis is supported by the evidence that the normal endometrial stroma expresses α SMA in the secretory phase.¹¹ Anyway, the stroma of APA does not seem to share the immunohistochemical alterations of the glandular component, and also the Ki67 L.I. is lower.^{11,24,25} This finding could differentiate APA from other more aggressive mullerian tumors in which stroma is an active component of the neoplasm. In particular, adenosarcoma shows significant increase of the Ki67 L.I. in the periglandular stroma, which is positive for CD10.²⁵

CD10 was also proposed as a diagnostic marker to differentiate APA from myoinvasive endometrial cancer. In fact, the latter one may show a CD10-positive fringe-like area surrounding neoplastic myoinvasive glands, which is absent in APA. However, this pattern was inconsistent and might be observed in as little as 5% of glands.^{22,23} In contrast, h-caldesmon appeared as a highly valuable diagnostic marker, as it was always diffusely positive in the infiltrated myometrium.^{23,27}

Strengths and Limitations

To the best of our knowledge, this is the first systematic review that assessed immunohistochemical features of APA. We evaluated the immunohistochemical markers separately for the different components of APA, discussing their pathogenic significance and their relation with AEH and with the 4 TCGA molecular groups of endometrial cancer. Moreover, we dealt with the possible application of immunohistochemistry in the differential diagnosis of APA.

The main limitation to our results lies in the rarity of APA, which results in a relatively small sample size. Moreover, the low number of molecular analyses and the lack of correlation with the prognosis might limit our results. Furthermore, some included studies showed limits in the presentation of data, as discussed in the "risk of bias assessment" results.

CONCLUSIONS

Immunohistochemically, APA appears as a variant of AEH with constant strong expression of hormone receptors and nuclear expression of β -catenin; this suggests that most APAs might be precursors of a subset of “copy number-low” endometrial cancer, characterized by *CTNGB1* mutation and a different prognosis.

APA morules appear as an “inert” component, which may derive from a prodifferentiative mechanism. On the basis of their peculiar immunophenotype, they can be differentiated from both a conventional squamous metaplasia and a solid tumor growth. In contrast, APA morules do not differ from morular metaplasia in other endometrioid proliferations.

The myofibromatous stroma of APA is similar to that of nonatypical adenomyoma; it may derive from endometrial stroma through a metaplastic, hormone-driven process. The low Ki67 L.I. may exclude a more aggressive mullerian tumor such as adenosarcoma. Negativity for h-caldesmon might be highly reliable for excluding a myoinvasive cancer; moreover, the latter one may show an inconstant fringe-like CD10 pattern, which is absent in APA.

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