Research Article

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Endometrial Tumors with "Shadow Cells" Intimate a Neoplastic Potential for Morules or Their Progenitors in β-Catenin Mutated Endometrial Adenocarcinomas.

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ABSTRACT

Background : Several features are shared by endometrial morules and pilomatrixoma-like endometrial carcinomas including CDX-2 and β-catenin nuclear staining, absence of p63 and p40 nuclear staining, and a unique form of end-epithelial differentiation that produces so-called shadow cells. Both endometrial morules and pilomatrixoma-like endometrial carcinomas may be associated with low-grade endometrioid adenocarcinoma.

Methods : We present 11 fully staged endometrioid endometrial adenocarcinomas that showed extrauterine metastases (Stages III and IV), morules, shadow cell differentiation, and nuclear β-catenin positive immunohistochemical staining (both in morules and in adjacent malignant glandular cells).

Results : All intrauterine adenocarcinomas had at least focal components of grade 1 endometrial adenocarcinoma and morules and showed nuclear β-catenin immunostaining in morules and in adjacent malignant glandular cells. 10 cases were stages III and IV endometrial cancers, and one, initially diagnosed as grade 1 stage II endometrioid endometrial adenocarcinoma, rapidly recurred in the retroperitoneum as a nuclear β-catenin positive pilomatrixoma-like tumor without an obvious glandular component. Two positive lymph node dissections showed metastatic adenocarcinoma with malignant glands, morules and shadow cells. Five cases showed at least focal components of morphologically divergent high-grade tumor with foci resembling malignant pilomatrixoma. Extrauterine "shadow cell granulomas" were detected in 8 cases.

Conclusion : Metastatic nuclear β-catenin positive endometrial adenocarcinomas with morules can metastasize and show pilomatrixoma-like tumor foci. This highlights the ability of these cancers to evolve into "other-than-glandular" carcinomas. Furthermore, because of their shadow cell features, we speculate that endometrial morules are not simply inert "epitumoral" elements; but that either they or the population of progenitor cells from which they eventuate harbors an abnormal β-catenin gene that may endow endometrial adenocarcinomas with morules the capacity for progressive, divergent differentiation into a higher-grade matrix-producing tumor.

INTRODUCTION

Endometrial morules have been traditionally viewed as a peculiar benign metaplastic change differing morphologically and immunophenotypically from conventional direct-from-gland squamous differentiation. Many pathologists consider them of no consequence. Endometrial morules show syncytial sheets of bland cells with round, ovoid or spindle-shaped nuclei, typically lacking keratinization, intercellular bridges, and prominent cell membranes (which are distinctive morphological features of squamous differentiation). Immunohistochemically, endometrial morules show nuclear β-catenin (due to mutation of the β-catenin gene), CDX2, and CD10 immunohistochemical positivity, and lack p63 and p40 expression (which are markers of usual-type squamous differentiation). Endometrial morules share these features with pilomatrixoma and other "matrix-producing" tumors.

The nuclear β-catenin accumulation and shadow cell keratinization of endometrial morules suggests a similarity with hair and/or odontogenic matrix-producing tumors (with some experts arguing for greater morphological and immunophenotypic overlap with adamantinomatous craniopharyngiomas than pilomatrixomas). [1] Morules show progressive end-epithelial differentiation with the eventual accumulation of shadow cells, as illustrated in **Image1**. Nuclear β-catenin is present in morules, focally in the epithelium of adjacent glandular cells, and in the developing shadow cell component, and its appearance is accompanied by nuclear CDX2 staining, as illustrated in **Image 2**. Some experts consider shadow cell differentiation of endometrial morules and direct from gland epidermal keratinization as

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different end-epithelial differentiation endpoints. [2, 3]

Endometrial morules are common components of premalignant glandular lesions that are generally followed by glandular, rather than squamous, carcinomas. Endometrial intraepithelial neoplasia with morules has neoplastic glands with significant estrogen and progesterone receptors and high mitotic activity, whereas accompanying morules are devoid of sex hormone receptors and have extremely low-proliferation rates. Some experts liken morules to a hormonally incompetent subpopulation of endometrial glandular lesions. Then again, when mutated, the same PTEN mutation is present in both the morular and glandular components of the neoplastic cells, indicating that neoplastic glandular elements and morules share a common progenitor[4].

Scheck et al. [5] reported a metastatic endometrial adenocarcinoma that mimicked pilomatrixoma of the distal vagina, citing an earlier report of a cutaneous metastasis in the upper limb derived from an ovarian endometrioid carcinoma that also mimicked pilomatrixoma [6], and several years later a second similar report surfaced. [7]. Scheck et al. reported a 55-years old woman with a history of FIGO stage IA grade 2 endometrioid endometrial carcinoma treated with laparoscopic hysterectomy and bilateral salpingo-oophorectomy who, at routine 3-year follow-up, reported occasional vaginal and rectal bleeding and on examination showed a tumor of the distal posterior wall of the vagina and introitus. Unfortunately, images of the original endometrial adenocarcinoma were not provided. Authors cautioned that the diagnosis of pilomatrixoma (a tumor primarily diagnosed in people under age 20 that occurs most often on the head, neck, arms, torso, or legs) should be made with caution in postmenopausal women, particularly in sites where pilomatrixoma is either uncommon or not known to occur. [5]

Weisman et al presented a series of grade 3 uterine endometrioid adenocarcinomas that showed striking morphologic and immunophenotypic likeness to tumors with shadow cells (such as malignant pilomatrixoma) that were accompanied by diffusely aberrant nuclear and cytoplasmic β-catenin and CDX2 expression (with the endometrioid components retaining PAX8 and ER expression but often also showing focally aberrant β-catenin and CDX-2 expression). All their tumors included at least focal components of grade 1 endometrial adenocarcinoma and, in their “case 1”, this component also showed morules. They interpreted their tumors as an aggressive CTNNB1-mutated subset of the “no specific molecular profile” (NSMP) category of endometrioid adenocarcinomas that demonstrated a form of high-grade divergent differentiation resembling, in their opinion, malignant cutaneous pilomatrixoma. Furthermore, their findings highlighted the difficulty in recognizing this “squamous-like” phenotype of endometrial adenocarcinoma at distant metastatic sites (given the consistent loss of ER and PAX8 expression and simultaneous gain of nuclear β-catenin and CDX2 expression). [8]

Image 1

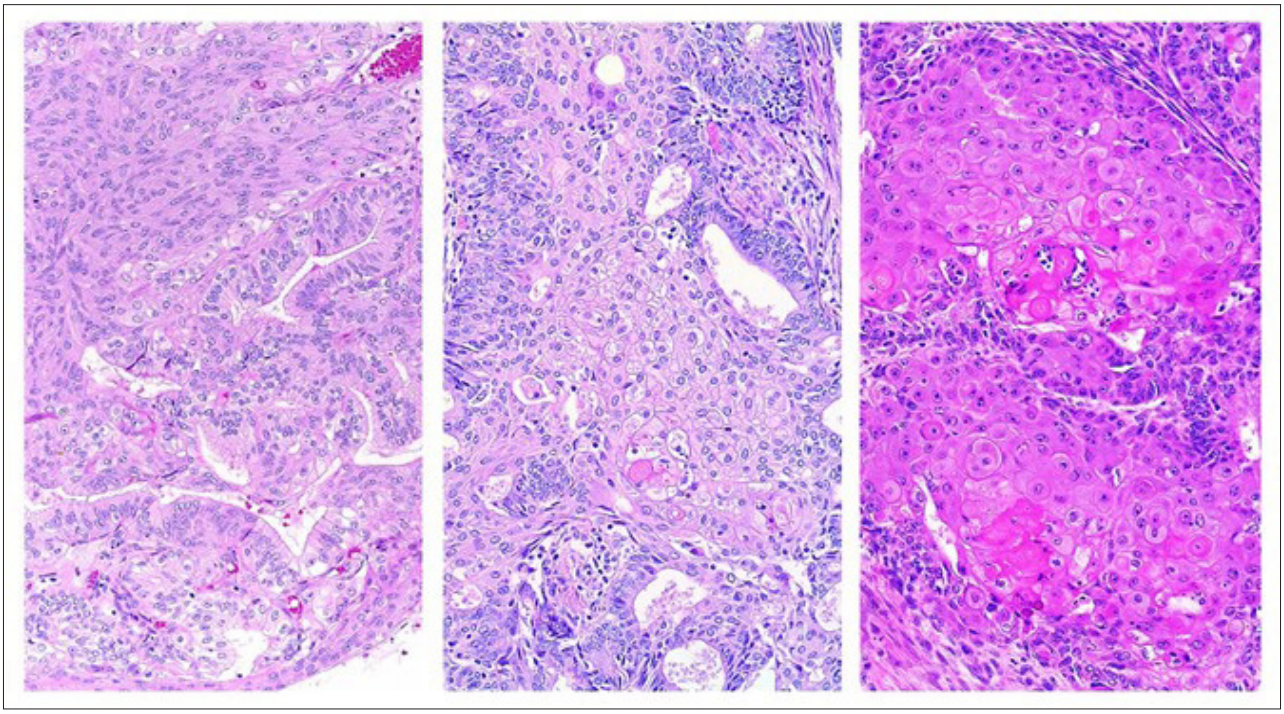


Image 1: Shadow or ghost cells are the products of end-epithelial differentiation in endometrial morules. This image shows morules in progressive stages of shadow cell accumulation. Shadow cell differentiation is a form of epithelial differentiation that is shared by tumors of hair and teeth. Shadow cells can be seen with pilomatrixomas, calcifying odontogenic cysts and craniopharyngiomatious adamantinomas.

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Image 2

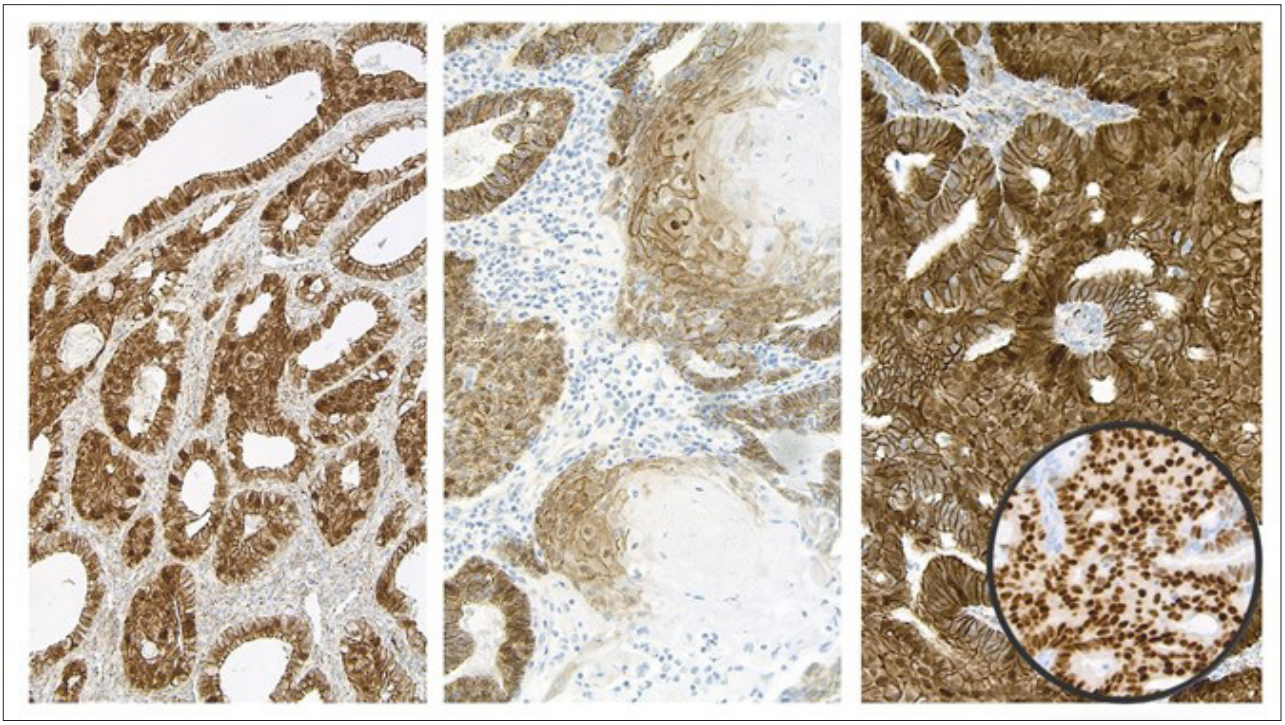


Image 2: β-catenin immunostaining of endometrial morules and their shadow-cells. In the left image, nuclear β-catenin nuclear immunostaining is seen in endometrial morules and extends to adjacent glandular epithelium. In the middle image, both morules and their derived shadow cells show nuclear staining for β-catenin. In the right image, there is concordant CDX2 (inset) and β-catenin nuclear staining. CDX2 is not behaving as a homeobox gene in this situation but correlates with nuclear β-catenin expression.

MATERIAL AND METHODS

We report 11 uterine endometrioid endometrial adenocarcinomas with extrauterine tumor metastases that showed morules, shadow cell differentiation, and nuclear β-catenin positive immunohistochemical staining (both in morules and in adjacent usual-type endometrioid adenocarcinoma). Data were recorded for tumor grade, stage, size, percent invasion, vascular invasion, cervical invasion, distant metastasis, lymph node status, ER quartile (0-25%, 26-50%, 51-75%, and 76-100% nuclear staining), PR quartile, p53-mutation status, PTEN-status, MMR-status, the presence of an extrauterine undifferentiated (pilomatrixoma-like) component, and/or shadow cell cells (including shadow cell granulomas).

Tumor immunohistochemistry

We report estrogen (ER) and progesterone (PR) receptor on a ranked (absent-to-strong immunodecoration) scale and semi-quantitatively assign the percent of positive cells into quartiles as 0-25%, 26-50%, 51-75%, and 76-100% nuclear staining. Otherwise, ASCO/CAP ER and PR guideline recommendations are used. [9] We evaluate the strongest-staining low-power field using a 4x-lens for stain-scoring. We report PTEN based on "all or none" staining (using an on-slide EIN control) as having either deleted or wild-type pattern. For MMR staining we evaluate MLH1, MSH2, MSH6, and PMS2, and any staining of tumor cell nuclei is reported as positive. All our cases showed a wild type p53 immunohistochemical staining pattern.

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CASE FINDINGS

Table 1. Eleven completely staged endometrial adenocarcinomas that had extrauterine metastases (Stages III and IV and a stage II tumor that recurred soon after surgery) and showed morules, shadow cell differentiation, and nuclear β-catenin positive immunohistochemical staining.

Tumor grade	Stage	Size mm	Percent invasion	Vascular invasion	Cervical invasion	Distant metastasis	Positive lymph nodes	LN dissections	ER quartile	PR quartile	p53-mutation	PTEN-wild-type	MMR-defective	Pilomatrixoma-like tumor	Shadow cell granulomas	Extrauterine shadow cells
3	IVB	100	95%	+	+	+	0	0	3	3	0	0	0	+	+	+
3	IVB	120	>50%	+	+	+	0	0	4	1	0	0	0	+	+	+
2	IVA	41	62%	+	+	+	0	0	4	4	0	0	M	+	+	+
1	IVB	30	59%	0	0	+	0	0	4	4	0	0	0	0	+	+
1	IIIC1	39	94%	0	0	0	+	+	4	4	0	0	0	+	+	+
1	IIIC1	100	88%	+	0	0	+	+	4	4	0	0	0	0	+	+
2	IIIB	30	100%	+	+	0	0	+	2	2	0	0	0	0	0	+
2	IIIB	36	100%	+	+	0	0	0	4	4	0	0	0	0	+	+
1	IIIA	28	43%	0	0	0	0	+	4	4	0	0	0	0	+	+
2	IIIA	75	75%	+	0	0	0	+	4	1	0	0	0	0	0	+
1	II*	40	81%	+	+	0	0	+	4	4	0	0	0	+	0	+

ER/PR quartile nuclear scoring: 0-25% = 1, 26-50% = 2, 51-75% = 3, and 76-100% = 4,For MMR-defective, M = MLH1 promoter hypermethylation

*Stage II tumor that showed early retroperitoneal tumor recurrence

In table 1, we present 11 completely staged endometrial adenocarcinomas that had extrauterine metastases (Stages III and IV and a stage II tumor that recurred soon after surgery) and showed morules, shadow cell differentiation, and nuclear β-catenin positive immunohistochemical staining (both in morules and in adjacent usual-type endometrioid adenocarcinoma). All the intrauterine tumors had at least focal components of grade 1 endometrial adenocarcinoma with morules. All extrauterine tumor metastases showed shadow cells with extrauterine shadow cell granulomas in 8 cases, and foci of high-grade pilomatrixoma-like carcinoma in 5 cases. Ten cases originated from stages III and IV endometrial cancers, and one from an initially diagnosed grade 1 stage II endometrial adenocarcinoma that recurred in the retroperitoneum as a largely undifferentiated neoplasm showing shadow cells, nuclear β-catenin, and was initially misdiagnosed as metastatic poorly differentiated squamous cell carcinoma.

Average uterine tumor measured 58 mm and there was, on average, 80% tumor invasion of the uterine wall. Eight cases (73%) showed vascular invasion in the uterus and six (55%) showed cervical stromal invasion. Four cases (36%) showed distant metastatic tumor and 2 of 6 lymph node dissections showed nodal metastases. All glandular tumor components were ER positive and 10 of 11 were PR positive. The average ER/PR quartile assignment was 3.7/3.1. All tumors showed wild type p53 nuclear staining and all were PTEN deleted. Ten cases probably represented NSMP tumors, and one was MMR-defective (MLH1/PMS2 deleted with increased MLH1 promoter hypermethylation).

Extrauterine metastatic tumors presented with foci showing endometrial carcinomas with shadow cells, (Image 3 [left]) morules (Image 3 [middle]), and anuclear, frequently granulomatous shadow cell aggregates involving serosa and omentum (**Image3** [right]). The undifferentiated tumor foci often bore little or no morphological resemblance to the index intrauterine tumor (**Image 4**). Two metastatic stage IVB tumors were evaluated with targeted next generation sequencing, and both confirmed CTNNB1 (exon 3) mutations.

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Image 3

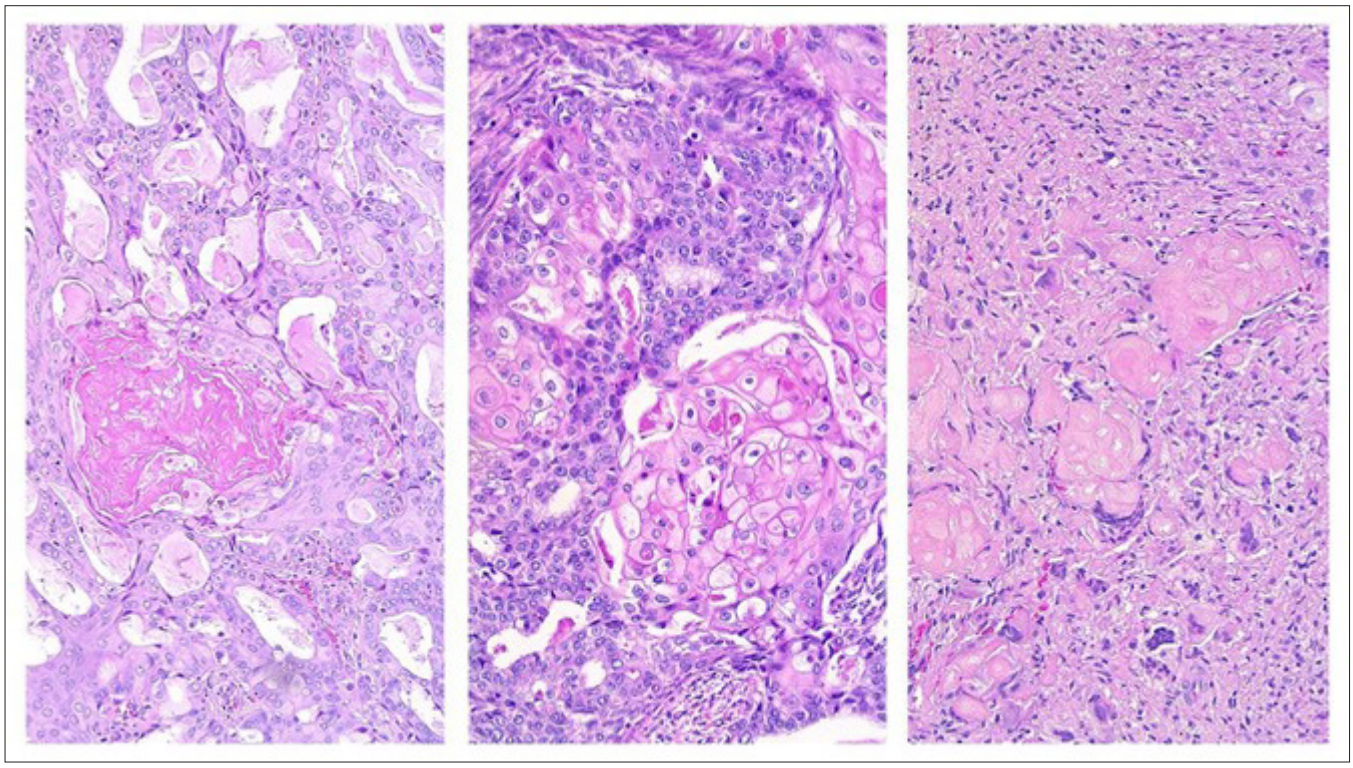


Image 3: Extrauterine shadow cells. This image shows ovarian (left image) and lymph node (middle image) metastases from endometrioid endometrial adenocarcinoma with morules as well as collections of shadow cells inciting a granulomatous response in the omentum (right image). When shadow cells overwhelm glandular cells, metastases can be mistaken for those of squamous cell carcinoma.

Image 4

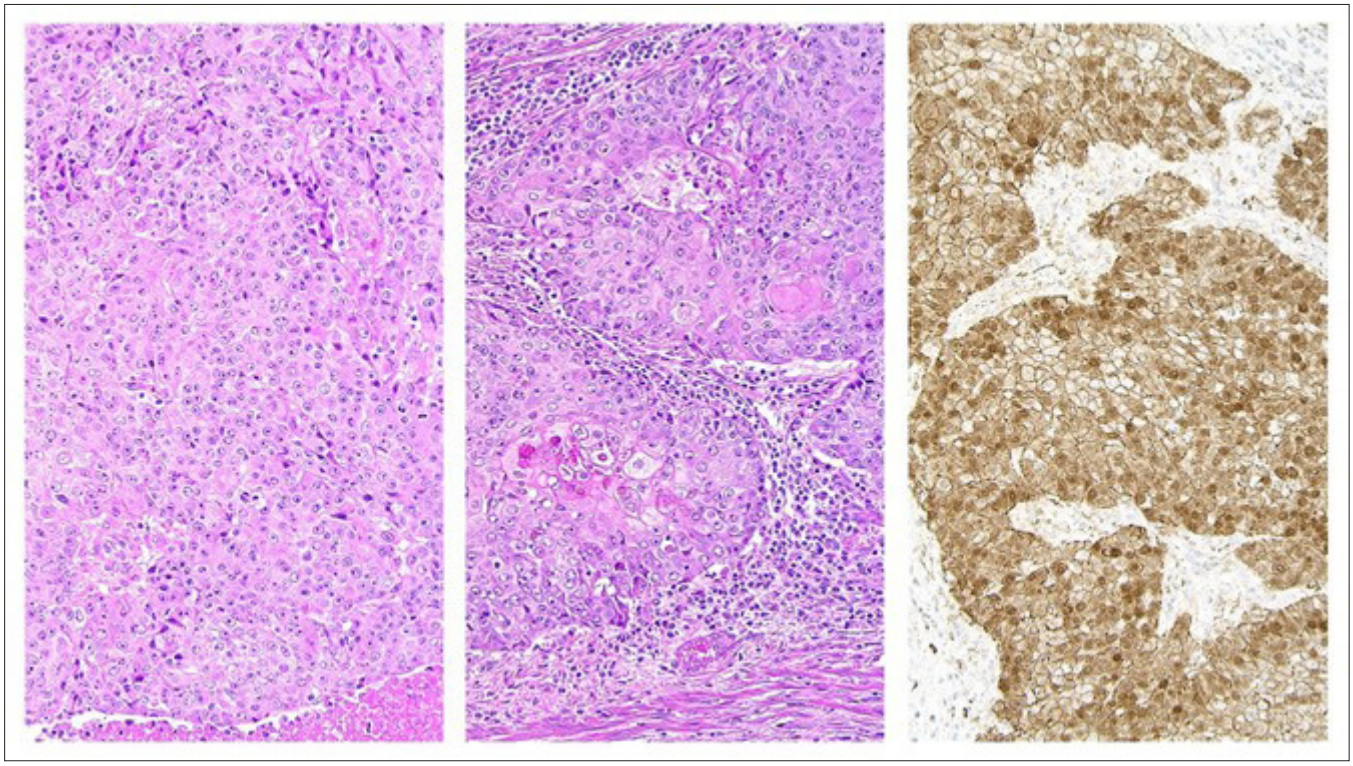


Image 4: An extrauterine metastasis from a grade 2 stage IVA endometrial adenocarcinoma that is, for a large part, undifferentiated. Shadow cells are seen in the middle image and nuclear β-catenin staining is seen in the right image. Weisman et al described similar tumors with ghost cells that were accompanied by nuclear β-catenin expression and contained foci of low-grade endometrial adenocarcinoma. They warned of the difficulty in recognizing this "squamous-like" phenotype at distant metastatic sites. [8]

DISCUSSION

Common to these tumors is nuclear β-catenin staining that is taken as evidence of a β-catenin gene mutation. β-catenin plays an important role in cell-to-cell adhesion, but apart from being an adherent junction protein, also has an important role in the wnt signal transduction pathway that regulates cell proliferation and differentiation.

β-catenin mutated tumors usually occur in younger women, tend to have low-grade histology, low levels of myometrial invasion, and low rates of lymphovascular space invasion—all traditional indicators of "good prognosis". Paradoxically, β-catenin mutated endometrial cancers have poor consequences with a significantly increased rate of disease recurrence and lower overall survival when compared to other grade, age, and stage-matched tumors. A summary of published data on clinical outcomes of patients with CTNNB1 (exon 3)/β-catenin mutations indicates that, since they show a worse recurrence free survival, β-catenin mutations may be prognostic for distant metastasis, leading some experts to conclude that they can be considered a fifth TCGA (The Cancer Genome Atlas) molecular subgroup. That is, a poor prognosis break-away group from NSMP tumors. [10, 11] Costigan et al. studied 39 CTNNB1 mutated endometrioid endometrial adenocarcinomas and 40 CTNNB1 wild type adenocarcinomas that were identified from a cohort of previously sequenced endometrial carcinomas using a targeted next-generation sequencing panel and found that nuclear β-catenin expression is significantly correlated with CTNNB1 (exon 3) mutation, that recurrence in patients with stage IA disease at diagnosis is higher in patients whose tumors are CTNNB1 mutated compared with CTNNB1 wild type, and that all recurrent tumors harboring exon 3 mutations and are histologically low grade (5 grade 1, 2 grade 2). [12]

Niu et al. examined the association of morules and squamous differentiation with CTNNB1 mutations and found a strong positive association between morules and glandular β-catenin nuclear staining (near-perfect agreement was reported for morules and glandular β-catenin nuclear staining in endometrioid intraepithelial neoplasia and endometrioid endometrial adenocarcinoma) whereas there was no association between morules and glandular PAX2 or PTEN aberrant expression or conventional squamous differentiation and aberrant expression of β-catenin, PAX2 or PTEN. Next-generation sequencing performed on 2 of their biopsies (pre- and post-treatment samples taken from one patient) showed an identical mutational profile in morules and glands—consistent with a shared neoplastic progenitor cell. Authors concluded that squamous differentiation and morules are distinct biological phenomena and that the presence of morules, but not squamous differentiation, is a reliable indicator of CTNNB1 mutations in both endometrioid endometrial adenocarcinoma and endometrioid intraepithelial neoplasia. [3]

Although previously reported, uterine endometrioid adenocarcinoma with pilomatrixoma-like areas had been thought to be uncommon among uterine cancers. [13] A limited number of cases were reported by Nakamura, who surmised that this form of epithelial differentiation may be more common than formerly estimated; and, in the past, probably shadow cells had been missed, ignored, or just recognized as conventional squamous metaplasia in the routine diagnostic practice. [14] Zámečník et al. reported that shadow cells are not uncommon in uterine endometrioid carcinoma, finding them in about 15% of 59 consecutively reviewed endometrioid adenocarcinoma cases. In their study, all endometrioid adenocarcinomas with shadow cells showed nuclear expression of β-catenin in areas of shadow cell differentiation and they proposed an operational role of the wnt signaling pathway with this morphological form of endometrioid tumorigenesis. Furthermore, the authors mentioned never having seen shadow cells in endocervical adenocarcinoma, proposing that finding shadow cells favors endometrial origin, further validating the conclusion of Weisman et al. [8] that, in the case of metastatic adenocarcinoma, nuclear β-catenin positive shadow cells are a clue to an endometrioid tumor primary. [15]

CONCLUSION

We conclude that the association of endometrial carcinomas with β-catenin positive morules and glands with extrauterine tumors featuring β-catenin positive pilomatrixoma-like foci points to the ability of the morule or of a common progenitor of glands and morules to evolve into a pilomatrixoma-like carcinoma. We posit this because our tumors shared morphological (shadow cell), immunohistochemical (β-catenin) and molecular (CTNNB1 (exon 3) mutation) features. The endometrial morules' consistent association with nuclear β-catenin in both morular and associated glandular epithelium makes them plausible signposts of pathological CTNNB1 mutations. [3] If endometrial adenocarcinoma with morules exists on a continuum with pilomatrixoma-like endometrial carcinoma, then, possibly, endometrial morules are not, as classically theorized, simply inert accompaniments of endometrial adenocarcinomas but that the subpopulation of tumor cells from which they eventuate may harbor an ability for progressive, divergent differentiation into high-grade matrix-producing carcinomas.

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