A Timely Update of Immunohistochemistry and Molecular Classification in the Diagnosis and Risk Assessment of Endometrial Carcinomas

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• Context.—Endometrial carcinoma is the most common gynecologic malignancy in the United States and has been traditionally classified based on histology. However, the distinction of certain histologic subtypes based on morphology is not uncommonly problematic, and as such, immunohistochemical study is often needed. Advances in comprehensive tumor sequencing have provided novel molecular profiles of endometrial carcinomas. Four distinct molecular subtypes with different prognostic values have been proposed by The Cancer Genome Atlas program: polymerase epsilon ultramutated, microsatellite instability hypermutated, copy number low (microsatellite stable or no specific molecular profile), and copy number high (serouslike, p53 mutant).

Objective.—To discuss the utilities of commonly used immunohistochemical markers for the classification of endometrial carcinomas and to review the recent advancements of The Cancer Genome Atlas molecular reclassification and their potential impact on treatment strategies.

Data Sources.—Literature review and authors' personal practice experience.

Conclusions.—The current practice of classifying endometrial cancers is predominantly based on morphology. The use of ancillary testing, including immunohistochemistry, is helpful in the identification, differential diagnosis, and classification of these cancers. New developments such as molecular subtyping have provided insightful prognostic values for endometrial carcinomas. The proposed The Cancer Genome Atlas classification is poised to gain further prominence in guiding the prognostic evaluation for tailored treatment strategies in the near future.

classifiers have been reported to more accurately reflect the underlying tumor biology and can provide independent prognostic and predictive information. Recent efforts have also been made to use immunohistochemical markers as surrogates for the TCGA classification. In practice, a combination of mismatch repair (MMR) protein and p53 immunohistochemistry is used to classify endometrial cancers into MMR-proficient or MMR-deficient (Figure 3), negative; +, focal positivity; ++, patchy positivity; ++++, strong diffuse positivity.

### Endometrioid Carcinoma

Endometrial endometrioid carcinoma (EEC) is the most common histologic type among endometrial carcinomas. It typically consists of glandular, cribriform, papillary, and microglandular growth patterns, with pseudostratified nuclei and variable nuclear pleomorphism (Figure 1, A through H). Low-grade EECs display diffuse strong positivity for ER/PR, negativity to patchy positivity for p16, and usually wild-type p53 staining pattern (Figure 1, C through F). High-grade (International Federation of Gynecology and Obstetrics [FIGO] 3) EECs may have invariably high nuclear grade, prominent macro- nucleoli, and brisk mitotic activity (Figure 3, A through H). In contrast to endometrioid carcinoma, 90% to 100% of EECs carry a TP53 mutation. In practice, p53 immunohistochemistry is an accurate surrogate for TP53 mutation, represented by 3 patterns of aberrant or mutation-type p53 staining, including overexpression (strong nuclear staining in at least 75% of tumor cells), null pattern (loss of staining in 100% of tumor cells) and cytoplasmic pattern (Figures 3, C, and 4, A through F). Approximately 90% of EECs are diffusely positive for p16 (Figure 3, D). They usually do not show aberrant expression of MMRs. Expressions of ER and PR in EECs are variable, ranging from negative to diffuse positive (Figure 1, E and F). Loss of PTEN and ARID1A expression is rare in EECs. Recent studies have shown that the addition of anti-HER2 therapy to standard chemotherapy improves progression-free survival of patients with HER2-positive advanced-stage and recurrent serous carcinoma.

In EEC, HER2 positivity is defined as an immunohistochemistry score of 3+ or 2+ with gene amplification by fluorescence in situ hybridization. A HER2 score of 3+ is assigned when intense complete or lateral/basolateral membranous HER2 immunostaining is present in more than 30% of tumor cells; a score of 2+ (equivocal staining) is assigned when intense complete or lateral/basolateral membrane staining is seen in 30% or less or weak to moderate staining is seen in 10% or more of tumor cells. Only tumors with 2+ HER2 immunohistochemistry score are subjected to fluorescence in situ hybridization, and an HER2:CEP17 ratio of 2 or higher, or an average of 6 or more HER2 signals per tumor cell, is considered HER2 amplified.

### Clear Cell Carcinoma

Endometrial clear cell carcinoma (CCC) is a rare type of endometrial cancer, accounting for less than 5% of all endometrial carcinomas. Clear cell carcinomas consist of proliferation of varying combinations of papillary, tubulocystic, and solid growth patterns (Figure 5, A through H).
Figure 1. Histology and immunohistochemistry of endometrial endometrioid carcinoma. Endometrial endometrioid carcinoma shows a glandular growth pattern (A and B), a wild-type p53 expression (C), patchy p16 staining (D), diffuse nuclear staining for estrogen receptor (E) and progesterone receptor (F), focal nuclear positivity for HNF-1β (G), and negativity for napsin A (H) (hematoxylin-eosin, original magnifications ×100 [A] and ×200 [B]; original magnification ×100 [C through H]).
These tumors are characterized by immunoreactivity for hepatocyte nuclear factor 1β (HNF-1β), napsin A, and α-methylacyl CoA racemase (AMACR), and negative staining for ER and PR (Figure 5, E through H). Notably, aberrant p53 expression is seen in one-third of CCCs. Patients with p53 mutated CCCs suffer from more aggressive clinical outcome compared with those without the mutation. MMR deficiency has been identified in 0% to 19% of CCCs. Loss of ARID1A is present in 13% to 22% of the tumors. Normal expressions of PTEN and CTNNB1 are usually seen, and rare cases with POLE mutation have been identified. Overall, the genetic findings suggest that the mutation profile of endometrial CCC is more serous-like than endometrioid-like.

MIXED CARCINOMA OF THE ENDOMETRIUM AND CARCINOSARCOMA

Endometrial mixed carcinoma is composed of 2 or more histologic subtypes of carcinomas with at least one falling in the type 2 category (serous or clear cell carcinoma). This mixed-type group represents up to 10% of endometrial cancers. Although the 2014 WHO classification requires each component to comprise at least 5% of the entire tumor, the presence of any percentage of a type 2 carcinoma component satisfies the diagnostic criterion for mixed carcinoma per the 2020 WHO classification. Recognition of mixed carcinoma is clinically relevant, and complete surgical staging should follow.

Carcinosarcoma/malignant mixed Müllerian tumor is a biphasic tumor composed of high-grade carcinomatous and sarcomatous components, accounting for up to 5% of endometrial carcinomas. Similar genetic mutations are shared between the 2 components, supporting that the sarcoma is derived from the carcinoma through epithelial-mesenchymal transition. For carcinosarcomas, 80% to 90% of cases carry TP53 mutation and about 67% carry PI3K pathway mutations.

UC AND DC OF THE ENDOMETRIUM

Undifferentiated carcinoma of endometrium is an aggressive subtype that represents about 2% of endometrial carcinomas. Histologically, UC consists of a sheetlike growth of discohesive cells that are monotonous and round or polygonal with scant cytoplasm, large vesicular nuclei, prominent nucleoli, and dense chromatin. No evidence of
Figure 3. Immunohistochemical markers for endometrial serous carcinoma. Endometrial serous carcinoma shows papillary and glandular patterns (A) with the tumor cells displaying high nuclear grade and prominent macronucleoli (B). It exhibits overexpression of p53 (C) with strong and diffuse p16 staining (D), variable positivity for estrogen receptor (E), negativity for progesterone receptor (F) and HNF-1β (G), and focal positivity for napsin A (H) (hematoxylin-eosin, original magnifications ×100 [A] and ×200 [B]; original magnification ×100 [C through H]).
lineage differentiation (solid growth without any pattern or glandular formation) should be found. Commonly seen in these tumors are prominent stromal infiltrating lymphocytes and brisk mitotic activity. Tumor necrosis may also be abundant.

Dedifferentiated carcinoma of endometrium is defined by the presence of 2 distinct carcinoma components: well-differentiated (FIGO 1 or 2) endometrioid adenocarcinoma and UC. The former is usually a mucosal lesion, whereas the latter is often deeply myoinvasive. The 2 components can vary in proportions, and the interface between the 2 can be abrupt or admixed. Irrespective of the amount of the undifferentiated component, DCs are far more aggressive than FIGO 2 endometrioid carcinomas. The undifferentiated component generally does not stain for epithelial markers (eg, cytokeratin [CK] AE1/AE3, CAM 5.1), E-cadherin, and gynecologic markers (eg, PAX8, ER, PR), in stark contrast to diffuse positivity of these markers in the endometrioid component.7,65 EMA and CK8/18 can be focally positive in the undifferentiated tumor cells.66 Neuroendocrine markers may stain scattered tumor cells (generally less than 10% of tumor cells). Interestingly, although morphologic and immunohistochemical features are significantly different, molecular analysis indicates that the undifferentiated component shares similar molecular alterations with the corresponding endometrioid component, suggesting a clonal evolution.64 About half of UC/DC cases show MMR deficiency64,66–70 and about 20% to 50% show aberrant expression of p53 in both components.64,67–71 Half to two-thirds of UC/DCs show switch/sucrose nonfermentable (SW1/SNF) complex inactivation, which may result in loss of expression SMARCA4/BRG1, SMARCB1/INI1, ARID1A and ARID1B in the UC component.67–69,71–74

OTHER TYPES OF ENDOMETRIAL CARCINOMA

Neuroendocrine neoplasms (NENs) are rare in the endometrium and often occur in association with other histologic types of endometrial carcinoma, mostly the endometrioid type.75 In the 2020 WHO classification, NENs in the female genital tract are classified as well-differentiated neuroendocrine tumors, that is, carcinoid tumor, and poorly differentiated neuroendocrine carcinomas (NECs), including small cell and large cell NEC.2 Endometrial NENs have similar histologic appearance to their counterparts in other organs. Poorly differentiated NECs are more prevalent than well differentiated neuroendocrine tumors in the uterine corpus.2 Most endometrial NENs are large cell NECs consisting of large polygonal cells having vesicular or

Figure 4. Expression patterns of p53 immunostaining. A, Normal wild-type pattern of p53 expression in an endometrioid carcinoma, showing variable intensities of tumor cell nuclei. B through D, Three abnormal p53 patterns in endometrial serous carcinomas. B, Overexpression of p53 in more than 75% of tumor cells. C, Null pattern with complete absence of p53 staining; note the wild-type internal control. D, Cytoplasmic pattern in tumor cells; note the p53 wild-type pattern in benign stromal cells (original magnification ×100).
Figure 5. Histology and immunohistochemical markers for endometrial clear cell carcinoma. Clear cell carcinoma displays a solid pattern with clear cytoplasm (A and B), a wild-type p53 pattern (C), strong and diffuse p16 staining (D), negativity for estrogen receptor (E) and progesterone receptor (F), positivity for HNF-1β (G), and focal positivity for napsin A (H) (hematoxylin-eosin, original magnifications ×100 [A and B] and ×200 [C through H]; original magnification ×100 [C through H]).
hyperchromatic nuclei and growing in organoid fashion (nests, trabeculae, or cords) with peripheral palisading. Mitotic figures in NECs are numerous, and geographic necrosis and hemorrhage are common. The 2014 WHO diagnostic criteria require more than 10% of the tumor cell to express one or more neuroendocrine markers (synaptophysin, chromogranin, and CD56), whereas the 2020 WHO version does not provide a clear cutoff. Some authors have proposed a 20% positivity cutoff to establish a diagnosis of NEN. Recent studies show that INSM1 seems to be a highly sensitive and specific neuroendocrine marker for the gynecologic tract. Rare tumor cells may stain positive for CD117 and TTF-1. Positive PAX8 immunostain is present in only a subset of the cases (33%).

Mesonephric-like adenocarcinoma is a rare type of endometrial carcinoma, representing about 1% of endometrial carcinomas. It is histologically similar to mesonephric carcinoma in cervix, but not associated with mesonephric remnants. Mesonephric-like adenocarcinoma is characterized by a variety of histologic architectures, including tubular, glandular, ductal, papillary, and solid growth patterns, but lacks squamous and mucinous differentiation. The classic pattern is tubules lined by cuboidal cells with eosinophilic colloidlike material within lumen. Most cases show variable positivity for GATA3, TTF-1, CD10 (apical/luminal staining), and PAX8, negativity for ER and PR, and a wild-type p53 pattern. Notably, GATA3 and TTF-1 can display an inverse staining pattern, which is a useful feature in limited biopsy specimens. The tumor cells also frequently show mutations of KRAS and PIK3CA and gain of 1q, and may show ARID1A mutation in a subset of tumors.

**GENERAL APPROACHES IN USING IMMUNOHISTOCHEMISTRY IN SUBCLASSIFICATION OF ENDOMETRIAL CARCINOMAS**

The diagnosis of low-grade endometrial carcinoma (FIGO 1 and 2 EECs) is usually not problematic. High-grade endometrial carcinomas, notably FIGO 3 endometrioid carcinomas, serous carcinoma, and clear cell carcinoma, may have overlapping histologic and immunohistochemical features that may impose significant diagnostic challenges (Figure 6).

A frequently encountered problem is the distinction of FIGO 3 EEC from ESC, for which an immunohistochemical panel of p53, p16, ER, and PR is generally helpful (Table; Figures 1, C through F, and 3, C through F). Briefly, combined aberrant p53 and strong/diffuse p16 staining along with patchy variable expression of ER and/or PR supports a diagnosis of serous carcinoma. Wild-type p53 and patchy p16 staining along with strong ER/PR expression favors a diagnosis of endometrioid carcinoma. In this basic panel, p16 is an essential marker for identifying p53 mutated EECs, as most EECs show variable patchy staining, with negative areas scattered throughout the tumor. For more difficult cases, for example tumors with aberrant p53 expression, patchy p16 staining, and ER and/or PR positivity, additional MMR, PTEN, and ARID1A immunostains may be pursued. Loss of expression of at least one MMR protein and negative PTEN or ARID1A expression favor a diagnosis of FIGO 3 EEC. Caution is needed in the interpretation of p16 in a small biopsy, as in limited tissue sampling, patchy p16 staining may be misread as diffuse/strong staining. If p53 staining is wild type, ESC should not be diagnosed unless the histomorphology is unequivocal for serous differentiation. Indeed, a small subset (approximately 5%) of ESCs harbor TP53 mutation but show a wild-type p53 immunostaining pattern. These tumors must be evaluated with a combination of morphologic assessment and extended immunohistochemical panel including MMR expression, PTEN, and ARID1A. Unfortunately, PTEN and ARID1A are not available in most pathology laboratories.

Although immunohistochemical studies play a limited role in the differential diagnosis of FIGO 3 EEC and CCC, a panel of HNF-1β, napsin A, AMACR, ER, and PR may be used to help with the distinction (Table; Figures 1, E through H, and 5, E through H). Clear cell carcinomas usually show negative staining for ER and PR and positive reactivity for HNF-1β, napsin A, and AMACR. Notably, HNF-1β is sensitive but suboptimally specific for CCCs, as it is also expressed in EEC and nonneoplastic endometrial glands during the secretory phase and with Arias-Stella reaction. AMACR is highly specific for CCC but not very sensitive. Napsin A is intermediate in terms of sensitivity and specificity between HNF-1β and AMACR. A combined positivity of these 3 markers may improve the distinction between CCCs and EECs.

For the distinction between serous and clear cell carcinomas, a panel of p53, p16, ER, PR, HNF-1β, napsin A, and AMACR can be used (Table; Figures 3, C through H, and 5, C through H). The diagnostic values of p53 and p16 are limited, as about 30% of clear cell carcinomas have aberrant p53 expression and strong p16 reactivity, although a wild-type p53 pattern has a high negative predictive value against the diagnosis of serous carcinoma. ER and PR are variably positive in ESC, in contrast to generally negative staining of the 2 markers in CCC. Either HNF-1β or napsin A seems to have significant performance in distinguishing CCC from ESC. For this purpose, both markers are comparable, and both are superior to AMACR. From a practical perspective, any 2 of the 3 markers may improve the identification of the CCC histotype. Lastly, loss of ARID1A suggests a diagnosis of CCC.

The differential diagnosis of DC includes carcinosarcoma/malignant mixed Mullerian tumor and FIGO 2 or 3 EEC. Unlike the latter entities, the undifferentiated component in DC shows focal positivity for EMA and CK8/18 and negativity for keratin AE1/AE3, E-cadherin, PAX8, ER and PR. Compared with carcinosarcoma, which usually contains high-grade carcinoma and pleomorphic spindle cell proliferation, DC has a low-grade gland-forming endometrioid carcinoma and an undifferentiated component of dyscohesive epithelioid cells that are negative for smooth muscle markers and epithelial markers.

The main considerations in the differential diagnosis of NEN include high-grade endometrioid adenocarcinoma, UC/DC, carcinosarcoma, and Ewing sarcoma/primitive neuroectodermal tumor. The histologic features on hematoxylin–eosin–stained sections are crucial for establishing the NEN diagnosis. Most NENs have a strong and diffuse positivity for one or more neuroendocrine markers, as opposed to focal staining in nonneuroendocrine tumors. It is worth noting that the diagnosis of NEN should rely largely on morphology rather than the cutoff value of neuroendocrine markers. For small cell carcinoma, neuroendocrine immunohistochemical stains are supportive, but not required. Notably, expression of one neuroendocrine marker in endometrial carcinomas is relatively frequent.
The loose terminology neuroendocrine differentiation is sometimes used in a pathology report to describe tumors that show neuroendocrine immunohistochemical expression. This terminology is to be avoided as it might cause confusion to clinicians. It is also recommended that in limited biopsy sample, neuroendocrine markers should be avoided unless there is clear evidence of neuroendocrine features, considering that the staining pattern may not represent the true nature of the whole lesion.

**TCGA CLASSIFICATION AND POTENTIAL INFLUENCE ON CLINICAL CARE**

The current system of risk assessment for endometrial carcinomas is based on clinicopathologic features, such as age, histologic subtype, tumor grade, and presence of lymphovascular space invasion. Survival has been found to correlate with the stage and histologic subtype of the diagnosis. Serous tumors or advanced-stage endometrial

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Figure 6. **Diagnostic algorithm for endometrial carcinomas.** Abbreviations: AMACR, a-methylacyl CoA racemase; ER, estrogen receptor; FIGO, International Federation of Gynecology and Obstetrics; H&E, hematoxylin-eosin; HER2, human epidermal growth factor receptor 2; HNF-1β, hepatocyte nuclear factor 1β; MMR, mismatch repair protein; PR, progesterone receptor; SW1/SNF, switch/sucrose nonfermentable.
cancers usually warrant adjuvant treatment. Patients with stage I or II disease usually have a more favorable prognosis than those with stage III or IV disease. However, the TCGA molecular classification identifies the patients who may have a different risk of recurrence from what is projected by traditional clinical risk-group assessment.

According to the TCGA molecular classification, FIGO 3 EECs can be stratified into 4 distinct subgroups with different prognostic implications: POLE-ultramutated, MMR deficient/hypermutated, p53 mutant/copy number high, and no specific molecular profile/copy number low. Patients with POLE-ultramutated EECs have the most favorable outcomes, which seems to supersede other prognostic factors such as high-grade disease. MMR-deficient EECs have an intermediate prognosis, whereas no specific molecular profile type carries an intermediate to excellent prognosis. The p53 mutated FIGO 3 EECs have a similar unfavorable survival to ESC, compared with their p53 wild-type counterparts.

It is noteworthy that TCGA molecular groups are also represented in UC/DC, with the MMR-deficient group appearing as the most common, followed by groups that behave like EEC3 p53 wild-type tumor and MMR-proficient p53 mutated cases that behave like ESC.

Further studies are needed to establish the prognostic significance of the TCGA classification in endometrial UC/DC. For carcinosarcomas, 60% to 78% are classified as high-grade disease. Molecular classification adds valuable prognostic information for subsets of endometrial carcinomas. Current practice and recommendations.}

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