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Immunohistochemistry for estrogen and progesterone receptors in the distinction of primary and metastatic mucinous tumors in the ovary: an analysis of 124 cases

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Estrogen (ER) and progesterone receptor (PR) expression in primary ovarian mucinous tumors and the utility of these markers for distinguishing metastatic mucinous carcinomas in the ovary from primary ovarian mucinous tumors have not been extensively investigated. Immunohistochemical studies were performed on 124 mucinous tumors, including 52 primary ovarian tumors (30 atypical proliferative (borderline) mucinous tumors of gastrointestinal type, 11 atypical proliferative (borderline) mucinous tumors of seromucinous (endocervical-like) type, and 11 invasive mucinous carcinomas of usual (gastrointestinal) type) and 72 metastatic mucinous carcinomas in the ovary (primary sites: colorectum (24), pancreas (13), endocervix (eight), stomach (four), gallbladder/bile duct (four), appendix (four), and unknown (15)). All atypical proliferative mucinous tumors of gastrointestinal type, primary ovarian mucinous carcinomas, and metastatic mucinous carcinomas were negative for ER and PR with the exception of three metastatic endocervical adenocarcinomas which exhibited only weak expression of ER without PR. All atypical proliferative mucinous tumors of seromucinous type expressed ER to some degree and seven had some expression of PR. Immunohistochemical assessment of hormone receptor expression is of no value in distinguishing the common types of primary ovarian mucinous tumors (atypical proliferative mucinous tumors of gastrointestinal type and mucinous carcinomas of usual type) from the vast majority of mucinous carcinomas metastatic to the ovary. The above observations on hormone receptor expression in primary ovarian mucinous tumors support the concept that atypical proliferative (borderline) mucinous tumors of gastrointestinal and seromucinous (endocervical-like) types are distinctive tumors.
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Among ovarian epithelial tumors, the mucinous tumors pose the greatest difficulty with regard to distinction of primary from metastatic tumors. The primary ovarian mucinous tumors, including the atypical proliferative (borderline) tumors and carcinomas, and metastatic mucinous carcinomas in the ovaries are usually easily distinguished when they exhibit characteristic gross and microscopic features. The primary tumors are typically large (usually greater than 15 cm), unilateral multicystic tumors with smooth capsules and are most often unassociated with extraovarian disease. The carcinomas most often arise in association with atypical proliferative (borderline) tumors and while they can exhibit destructive stromal invasion, they frequently display confluent glandular or expansile, rather than infiltrative, patterns of invasion. In contrast, typical features of metastatic mucinous carcinomas in the ovary that distinguish them from primary tumors include bilaterality, smaller size (often less

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than 10 cm), ovarian surface involvement, a nodular pattern of involvement, and an infiltrative pattern of stromal invasion.[1–3] However, some metastatic mucinous carcinomas can manifest one or more features suggesting a primary ovarian tumor, including presentation as a large unilateral tumor in the absence of a known extraovarian primary site and formation of a multicystic tumor exhibiting confluent glandular or expansile, rather than infiltrative, patterns of invasion, thus simulating primary ovarian atypical proliferative (borderline) mucinous tumors with intraepithelial carcinoma or well-differentiated mucinous carcinomas of confluent glandular type.[4–10] Despite recognition of the ability of these metastases to simulate primary ovarian tumors and recent studies providing refined diagnostic criteria for ovarian mucinous tumors,[1,2,11,12] the problem of distinguishing these tumors persists. This problem is compounded by the fact that in routine practice metastatic mucinous carcinomas are more common than primary ovarian mucinous carcinomas,[3] with those of gastrointestinal and pancreaticobiliary tract origin being the most commonly encountered types.[1,3]

When characteristic gross and microscopic features are lacking, ancillary studies are often necessary to distinguish primary ovarian mucinous tumors from metastases. Unfortunately, only a limited number of immunohistochemical markers has been shown to be useful in this distinction, including cytokeratins 7 and 20 and Dpc4. In addition, the utility of these markers is restricted based on the primary site of origin under consideration. Thus, cytokeratins 7 and 20 are useful for distinction of ovarian endometrioid and mucinous tumors from metastatic lower intestinal tract carcinomas (most colorectal and appendiceal carcinomas) but not from many other metastatic carcinomas (including pancreaticobiliary, gastric, endocervical, endometrial and pulmonary carcinomas), whereas Dpc4 is useful in the distinction of ovarian mucinous tumors from pancreatic carcinomas in only $\sim$50% of cases.[13–19] Owing to these limitations and restrictions, identification of additional markers to assist in the distinction of these mucinous tumors would be valuable. Primary ovarian epithelial tumors, particularly those of serous and endometrioid types, are known to often express hormone receptors.[16,20–32] Thus, one could speculate that other subtypes of ovarian epithelial tumors, such as the mucinous type, might also express estrogen receptor (ER) and progesterone receptor (PR) and that these markers might serve to distinguish primary and metastatic mucinous tumors in the ovary. Since data in the literature on expression of ER and PR in primary ovarian mucinous tumors and in mucinous carcinomas of various extraovarian sites is limited and conflicting,[16,20–27,33–51] we analyzed a large number of rigorously classified tumors to assess the utility of these markers in the distinction of these tumors.

Materials and methods

Case Selection

A total of 124 mucinous tumors involving the ovary were selected from the surgical pathology files of The Johns Hopkins Hospital and the Armed Forces Institute of Pathology (Department of GYN & Breast Pathology) from 1990 to 2005. In all, 87 tumors (31 primary, 56 metastatic) were routine in-house cases and 37 were consultation cases (21 primary, 16 metastatic). A total of 52 cases were primary ovarian mucinous tumors, including 30 atypical proliferative (borderline) mucinous tumors of gastrointestinal type, 11 atypical proliferative seromucinous tumors (mucinous borderline tumors of endocervical-like type), and 11 invasive mucinous carcinomas of usual type (referring to the 'common' type of ovarian mucinous carcinomas having gastrointestinal-type or nonspecific mucinous differentiation rather than the rare mucinous carcinomas of seromucinous (endocervical-like) type). In all, 72 cases were metastatic mucinous carcinomas involving the ovary, with primary sites including colorectum (24), pancreas (13), endocervix (eight), stomach (four), gallbladder/biliary tract (four), appendix (four), and unknown primary sites (15). The metastases classified as of 'unknown primary site' exhibited characteristic gross and microscopic features of metastases (with morphologic features, and in some cases immunohistochemical features, most often implicating the gastrointestinal tract as the primary site),[52] but a specific primary site had not been established in these cases. Clinical data, data from imaging studies, and pathologic (gross and microscopic) criteria[2] were used to rigorously classify the tumors as primary or metastatic.

Immunohistochemistry

Immunohistochemical stains were performed at both PhenoPath Laboratories and The Johns Hopkins Hospital using formalin-fixed, paraffin-embedded tissue sections. The manufacturer, clone, dilution, and pretreatment details for each primary antibody are summarized in Table 1. Unstained sections were deparaffinized and rehydrated prior to subjecting them to antigen retrieval using protocols optimized for each antibody.

The immunohistochemical method used at PhenoPath Laboratories was performed as follows. Following pretreatment, primary antibodies (ER and PR) were applied to respective sections and incubated for 30 min at room temperature. SP-1 and SP-2 rabbit monoclonal antibodies for ER and PR, respectively, were employed because preliminary evidence suggests that they are more sensitive than the mouse monoclonal antibodies.[53,54] Antibody localization was achieved by incubating slides for 30 min at room temperature in Envision + -labeled polymer (DakoCytomation, Carpinteria, CA, USA)

Table 1 Details of immunohistochemical analysis

Antibody	*Manufacturer*	*Clone*	*Dilution*	*Pretreatment*
ER[a]	NeoMarkers	SP-1	1:500	Microwave pressure cooker, 8 min, 10 mM citrate buffer (pH 6.0)
ER[b]	Ventana	6F11	Prediluted	CC1 antigen retrieval solution (Ventana) (prediluted; pH 8.0), performed on autostainer, 95°C, 30 min
PR[a]	NeoMarkers	SP-2	1:500	Microwave pressure cooker, 8 min, 10 mM citrate buffer (pH 6.0)
PR[b]	Ventana	16	Prediluted	CC1 antigen retrieval solution (Ventana) (prediluted; pH 8.0), performed on autostainer, 95°C, 30 min

[a]PhenoPath Laboratories.

[b]Johns Hopkins Hospital Immunohistochemistry Laboratory.

Table 2 Distribution of expression of ER and PR in primary ovarian mucinous tumors

	ER[a]					*PR*[a]				
	0	*1+*	*2+*	*3+*	*4+*	*0*	*1+*	*2+*	*3+*	*4+*
Atypical proliferative mucinous tumors, gastrointestinal type ($n=30$)	30 (100%)	0	0	0	0	30 (100%)	0	0	0	0
Atypical proliferative seromucinous tumors ($n=11$)	0	2 (18%)	2 (18%)	2 (18%)	5 (45%)	4 (36%)	3 (27%)	1 (9%)	1 (9%)	2 (18%)
Invasive mucinous carcinomas ($n=11$)	11 (100%)	0	0	0	0	11 (100%)	0	0	0	0

[a]Staining distribution (percentage positive cells): 0: ≤5%; 1+: 6–25%; 2+: 26–50%; 3+: 51–75%; and 4+; 76–100% (all reactions exhibited moderate to strong staining intensity).

using a Dako autostainer. The slides were then incubated for 10 min at 37°C in a solution containing 3% hydrogen peroxide and 3,3′-diaminobenzidine.

The immunohistochemical method used at The Johns Hopkins Hospital laboratory was performed as follows. Following pretreatment, primary mouse monoclonal antibody (ER and PR) was applied to respective sections and incubated for 32 min (ER) and 16 min (PR), respectively, at room temperature. Antibody localization was achieved by incubating slides for 16 min at room temperature in *i*VIEW-labeled conjugate (Ventana, Tucson, AZ, USA) using a Ventana autostainer (Benchmark XT). The slides were then incubated for 8 min at room temperature in a solution containing hydrogen peroxide (prediluted; Ventana) and 3,3′-diaminobenzidine.

Interpretation and Scoring of Immunohistochemical Preparations

Reactions were interpreted as positive based on nuclear staining. Immunohistochemical results were scored based on the percentage of cells showing expression: negative: ≤5%; and positive: >5%. For descriptive purposes, the distribution of staining was semiquantitatively scored based on the percentage of positive cells: 0: ≤5%; 1+: 6–25%; 2+: 26–50%; 3+: 51–75%; and 4+; 76–100%. Intensity of staining was noted but not used for scoring. Although immunohistochemical stains were performed in two different laboratories, tumors of the same type showed no notable differences in staining profiles between the laboratories.

Results

Primary Ovarian Tumors (Table 2)

Among all primary ovarian mucinous tumors evaluated, hormone receptor expression was restricted to the seromucinous tumors. In all, 11 (100%) atypical proliferative (borderline) mucinous tumors of seromucinous-type expressed ER (Figure 1). The immunohistochemical score was 1+ in two cases, 2+ in two, 3+ in two, and 4+ in five. The intensity of expression was moderate to strong. Seven of 11 (64%) seromucinous tumors expressed PR (Figure 1). The immunohistochemical score was 0 in four cases, 1+ in three, 2+ in one, 3+ in one, and 4+ in two. The intensity of expression was moderate to strong. Neither ER nor PR was expressed in 30 atypical proliferative mucinous tumors of gastrointestinal type (Figure 2) or in 11 invasive mucinous carcinomas. ER and PR expression were observed in normal ovarian stroma which served as an internal positive control.

Metastatic Mucinous Carcinomas Involving the Ovary (Table 3)

Three of eight (38%) endocervical adenocarcinomas expressed ER (Figure 3). The immunohistochemical

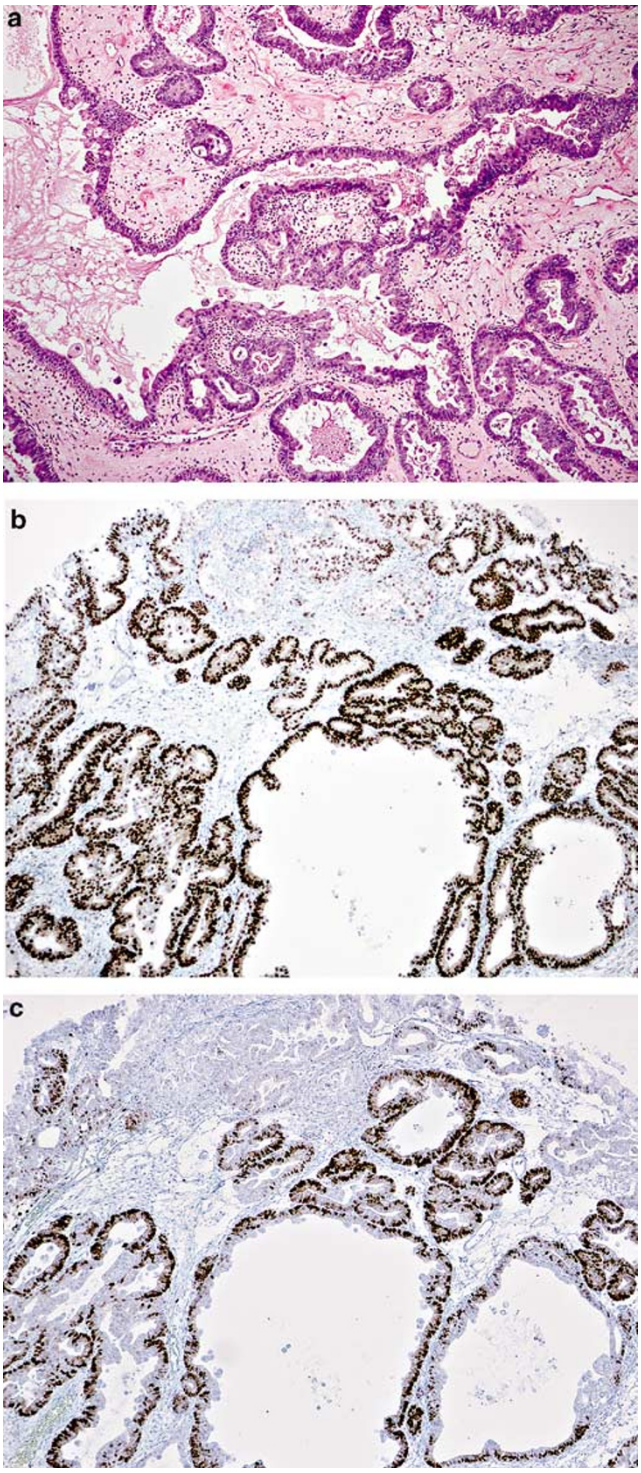

Figure 1 (**a**) Atypical proliferative (borderline) tumor of seromucinous (endocervical-like) type. (**b**) Tumor exhibits expression of ER (immunohistochemical score for the entire tumor was 4+, with moderate to strong staining intensity). (**c**) Tumor exhibits expression of PR (immunohistochemical score for the entire tumor was 3+, with moderate to strong staining intensity).

score was 0 in four tumors, 1+ in one, 2+ in one, and 4+ in one. The intensity of expression was uniformly weak in those showing expression. PR was not expressed in any of these eight tumors.

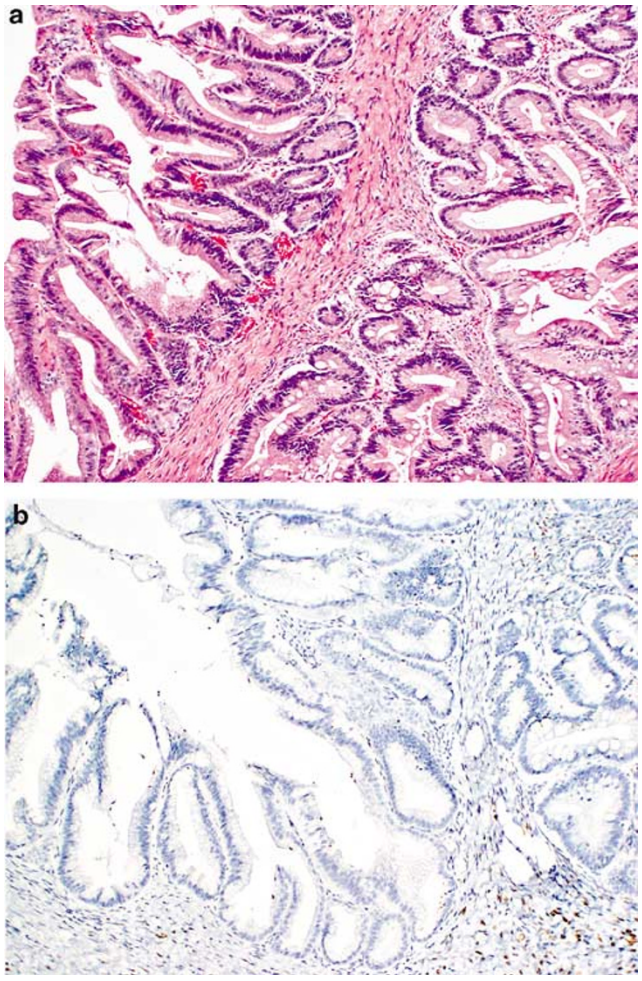

Figure 2 (**a**) Atypical proliferative (borderline) mucinous tumor of gastrointestinal type. (**b**) Tumor lacks expression of ER (ovarian stroma serves as internal positive control) and was also negative for PR (not shown).

Neither ER nor PR was expressed in the remaining metastatic tumors, including 24 colorectal adenocarcinomas, 13 pancreatic adenocarcinomas, four gastric adenocarcinomas, four gallbladder/biliary tract adenocarcinomas, four appendiceal adenocarcinomas, and 15 metastatic adenocarcinomas of unknown primary site. In cases in which normal ovarian stroma was present, staining for ER and PR was present and served as an internal positive control.

Discussion

Among the metastatic mucinous carcinomas evaluated in this study, neither ER nor PR expression was observed in any metastases of gastrointestinal tract origin, including those metastatic from the colorectum, pancreas, biliary tract, gallbladder, and appendix, or in metastases of unknown primary sites which were most likely also of gastrointestinal tract origin based on morphologic features. In other

Table 3 Distribution of expression of ER and PR in metastatic mucinous carcinomas involving the ovary

Origin	*ER*[a]					*PR*[a]				
	0	*1+*	*2+*	*3+*	*4+*	*0*	*1+*	*2+*	*3+*	*4+*
Colorectal ($n=24$)	24 (100%)	0	0	0	0	24 (100%)	0	0	0	0
Unknown ($n=15$)	15 (100%)	0	0	0	0	15 (100%)	0	0	0	0
Pancreatic ($n=13$)	13 (100%)	0	0	0	0	13 (100%)	0	0	0	0
Endocervical ($n=8$)	5 (63%)	1[b] (13%)	1[b] (13%)	0	1[b] (13%)	8 (100%)	0	0	0	0
Gastric ($n=4$)	4 (100%)	0	0	0	0	4 (100%)	0	0	0	0
Gallbladder/biliary tract ($n=4$)	4 (100%)	0	0	0	0	4 (100%)	0	0	0	0
Appendiceal ($n=4$)	4 (100%)	0	0	0	0	4 (100%)	0	0	0	0

[a]Staining distribution (percentage positive cells): 0: $\leq$5%; 1+: 6–25%; 2+: 26–50%; 3+: 51–75%; and 4+; 76–100%.
[b]Weak staining intensity.

published studies, expression of hormone receptors in gastrointestinal and pancreaticobiliary adenocarcinomas varies markedly, with many studies demonstrating a lack of expression of ER and PR[16,21,22,25,27,34,36–38,41,45,46,48,50] but others reporting expression of these markers.[25,33,39,40,44,45,47–49,51,55] The reasons for the differing results in the latter studies are not apparent, but variation in immunohistochemical methods (including different antibodies and/or epitope retrieval methods), case selection, and tumor classification could be factors; however, the use of a more sensitive tyramine amplification technique accounts for increased apparent expression of ER in one study,[25] no ER expression was found in any colorectal adenocarcinomas in the largest of the series,[48] and our unpublished observations suggest that ER or PR expression in gastrointestinal and pancreatobiliary tract tumors is exceedingly rare.

The only metastases in our study exhibiting hormone receptor expression were a minority of metastatic endocervical adenocarcinomas, which demonstrated weak expression of ER without PR. This result is in keeping with other studies demonstrating a low frequency of hormone receptor expression in primary endocervical adenocarcinomas.[56–61] Metastatic endocervical adenocarcinomas in the ovary usually simulate either primary ovarian endometrioid tumors or mucinous tumors of gastrointestinal (not seromucinous/endocervical-like) type. The infrequent expression of hormone receptors in endocervical adenocarcinomas, lack of expression of hormone receptors in both primary ovarian atypical proliferative (borderline) mucinous tumors of gastrointestinal type and ovarian mucinous carcinomas of usual type in this study, and frequent expression of hormone receptors in ovarian endometrioid tumors observed in other studies[16,24,26,31] indicate that ER/PR expression is most often useful only in the distinction of endocervical adenocarcinomas from primary ovarian endometrioid but not mucinous tumors. It is important to note that the application of ER/PR expression to an individual case for which the differential diagnosis concerns metastatic endocervical adenocarcinoma *vs* a primary ovarian tumor must be performed with awareness that interpretation depends on the type of differentiation exhibited by the tumor (mucinous *vs* endometrioid) and recognition that some endocervical adenocarcinomas can retain expression of hormone receptors. Thus, other ancillary techniques (p16 expression and human papillomavirus DNA detection) may be necessary for definitive distinction of these tumors.[5]

Metastatic mucinous carcinomas from the breast, endometrium, and lung were not included in this study since we did not have any retrievable cases with paraffin blocks available in our files. These types of metastatic mucinous carcinomas are rather uncommon and usually do not enter into the differential diagnosis of mucinous tumors involving the ovary. Hormone receptor expression is frequently observed in breast carcinomas, including mucinous/colloid and signet ring cell types,[37,62–65] and mucinous carcinomas of the endometrium[66] and could serve to distinguish metastases of these tumors from the common types of primary ovarian mucinous tumors. In particular, hormone receptor expression is of value for distinguishing the signet ring cell variant of lobular carcinoma from metastatic gastric and appendiceal signet ring cell carcinomas, which lacked hormone receptor expression in our study. Lung adenocarcinomas are usually negative for ER and PR, but expression has been reported in some studies.[47,67,68]

Among the primary ovarian mucinous tumors in this study, ER and PR expression were observed exclusively in atypical proliferative (borderline) mucinous tumors of seromucinous (endocervical-like) type; atypical proliferative (borderline) mucinous tumors of gastrointestinal type and primary ovarian invasive mucinous carcinomas of the usual type did not express ER or PR. Previous studies have reported a low frequency of ER and PR expression in atypical proliferative (borderline) mucinous tumors of gastrointestinal type (0–14%)[26,43,69] and variable expression of ER and PR in primary ovarian invasive mucinous carcinomas (ER in 0–70%, PR

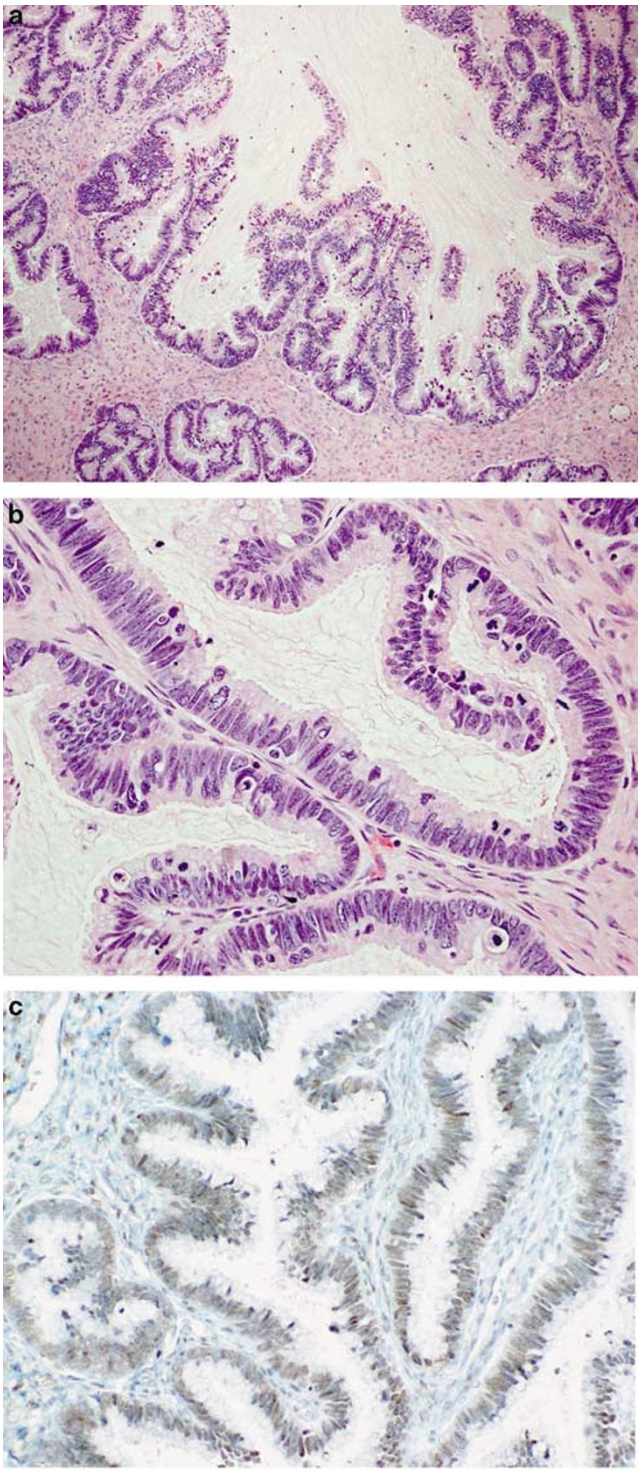

Figure 3 (**a** and **b**) Metastatic endocervical adenocarcinoma involving the ovary simulates an atypical proliferative (borderline) mucinous tumor of gastrointestinal type with intraepithelial carcinoma. The tumor lacks evidence of stromal invasion but exhibits notable nuclear atypia. Mucinous epithelium displays features characteristic of an HPV-related endocervical adenocarcinoma (nuclear atypia, numerous mitotic figures, and apoptotic bodies). (**c**) Tumor exhibits some expression of ER, which was uncommon among all tumors of this type (immunohistochemical score for the entire tumor was 4+, with uniformly weak intensity).

in 0–30%).[16,23,24,26,35,42,43,69] In contrast, uniform expression of ER has been reported in a very limited number of seromucinous (endocervical-like) tumors, including both atypical proliferative (borderline) tumors and the rare carcinomas of this type.[20,42] While ER expression can distinguish the relatively uncommon seromucinous tumors from most metastatic mucinous carcinomas, including most metastatic endocervical adenocarcinomas, seromucinous tumors are most commonly of the atypical proliferative type and are usually readily recognized as primary ovarian tumors due to their distinctive features.[70–73] Metastatic mucinous carcinomas exhibiting deceptive patterns of invasion (confluent glandular or expansile rather than infiltrative) in the ovary usually simulate the more common atypical proliferative mucinous tumors of gastrointestinal type and mucinous carcinomas of usual type. Thus, the lack of ER and PR expression in both the common primary ovarian mucinous tumors and most metastatic mucinous carcinomas (including all those of gastrointestinal origin, which are the most common type) demonstrates that hormone receptor expression is not useful for distinction of these mucinous tumors in the ovary.

The above observations on hormone receptor expression in primary ovarian mucinous tumors support the concept that atypical proliferative (borderline) mucinous tumors of gastrointestinal and seromucinous (endocervical-like) types are distinctive tumors.[74] Subclassification of these types based on morphology is occasionally problematic for pathologists who have limited experience with the uncommon seromucinous type, and thus, ER/PR expression may be useful in this regard. Shared expression of hormone receptors by atypical proliferative (borderline) serous and seromucinous tumors supports the notion that the seromucinous subtype is more closely related to the serous type than the gastrointestinal mucinous type and, in conjunction with morphologic features, justifies the designation 'seromucinous'.[73,74]

In summary, hormone receptor expression is of no value in distinguishing atypical proliferative (borderline) mucinous tumors of gastrointestinal type and primary ovarian invasive mucinous carcinomas of usual type from the vast majority of mucinous carcinomas metastatic to the ovary. Additionally, lack of hormone receptor expression in primary ovarian mucinous carcinomas of usual type suggests there is no role for hormonal therapy in the management of these tumors.

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