Utility of CDX-2 in Distinguishing Between Primary and Secondary (Intestinal) Mucinous Ovarian Carcinoma

An Immunohistochemical Comparison of 43 Cases

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Abstract: Primary and secondary mucinous tumors can involve the ovaries and have similar histologic appearances. The differential diagnosis is important for surgical and chemotherapeutic treatment and for the prognosis, but often it is extremely difficult. This article discusses an immunohistochemical panel that includes carcinoembryonic antigen (CEA), cytokeratin (CK) 7, CK20, CA125, CA19.9, and a new marker, CDX-2, for the distinction between primary ovarian mucinous carcinomas and metastatic (intestinal) ovarian tumors. Forty-three cases representing primary and secondary ovarian tumors were considered and consisted of 14 primary mucinous ovarian carcinomas (PMOCs) and 29 secondary (intestinal) ovarian tumors (SIOTs). Fisher exact test was performed to evaluate the reliability of the respective antibodies to discriminate between PMOCs and SIOTs. CDX-2 was diffusely positive in all SIOTs and was expressed focally in 3 cases (21.42%) of PMOCs. CK7 was diffusely positive in 13 cases (44.82%) of SIOTs and in 13 cases (92.85%) of PMOCs. CK20 was diffusely positive in 17 cases (58.62%) of SIOTs and in 6 cases (42.85%) of PMOCs. CEA was diffusely positive in 28 cases (96.55%) of SIOTs and in 12 cases (85.71%) of PMOCs. CA19.9 was positive in all SIOTs and in 12 cases (85.71%) of PMOCs. CA125 was positive in 3 cases (10.34%) of SIOTs and in 4 cases (28.57%) of PMOCs. CK7 and especially CDX-2, a specific and sensitive marker, can aid pathologists in making a differential diagnosis ($P = 0.003$ and $P < 0.0005$, respectively), whereas CEA, CK20, CA125, and CA19.9 markers are not high enough to distinguish between primary and secondary mucinous ovarian tumors.

Key Words: CDX-2, ovarian carcinoma, intestinal carcinoma, mucinous tumor, metastases

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Mucinous metastases to the ovaries are more frequent than primary mucinous ovarian carcinoma (PMOC).[1] Primary mucinous carcinomas appear to account for approximately 3.6% of ovarian epithelial neoplasms, 12% of ovarian mucinous neoplasms, and 11% of ovarian carcinomas.[2]

Ovarian mucinous carcinomas occur in patients in the 4th to 7th decades. As many as 63% of the cases are International Federation of Gynecology and Obstetrics (FIGO) stage I, and nearly all stage I cases have unilateral ovarian involvement.[3] Therefore, these women do not generally present signs and symptoms of abdominal carcinomatosis, but the clinical presentation is usually that of a large mass and abdominal distension. The proportion of stage I cases varies widely among studies because of the difficulty in distinguishing intraepithelial carcinomas from invasive carcinomas.[4]

Before making a diagnosis of mucinous carcinoma of the ovary, it is imperative to exclude metastatic mucinous carcinoma. Mucinous adenocarcinomas of the pancreas, biliary tract, colon, appendix, and cervix can mimic ovarian mucinous carcinomas.[5–8] Approximately 40% of adenocarcinoma metastases to the ovary originate from the colon.[9] It may be difficult to differentiate between primary and metastatic lesions according to clinical and histopathologic aspects, but the differential diagnosis is decisive for the surgical and chemotherapeutic treatment and for the prognosis.

Sometimes the clinical history of a patient can help in the exact diagnosis, but a negative anamnesis for neoplastic disease is not helpful, because sometimes metastases can anticipate a primary tumor. A recent report indicates a rule that correctly classified 90% of the neoplasm. It classifies all bilateral mucinous carcinomas as metastatic and unilateral mucinous carcinomas 10 cm or greater as primary.[10]

Cdx genes are homeobox genes necessary for intestinal organogenesis and encode for nuclear transcription factors involved in proliferation and differentiation of intestinal epithelial cells in fetal and adult tissue.[11] CDX-2 is expressed in normal colonic epithelia and most colorectal adenocarcinomas.[12,13]

The aim of the present study was to evaluate whether the immunohistochemical expression of CDX-2 in a series of mucinous ovarian carcinomas and a series of secondary ovarian carcinoma could be a reliable marker for identifying colorectal metastases from primary ovarian tumors and whether this marker could be added to an immunohistochemical panel of specific markers useful in this differential diagnosis.

MATERIALS AND METHODS

Case Selection

The files of the Department of Human Pathology and Oncology of the University of Florence were searched from 1990 to 2002 for the diagnosis of ovarian mucinous tumors.

From these neoplasias, 14 cases of primary mucinous carcinoma of the ovary and 29 cases of ovarian metastases from intestinal carcinoma were selected to be evaluated in the current analysis. Of these 43 cases, histologic material, clinical data, and follow-up were available. Clinical data available for each patient included age, previous medical history, surgical treatment, disease stage, adjuvant therapy if performed, and follow-up.

All microscopic slides were reviewed without knowledge of the previous medical history or the clinical outcome.

The age range of the patients with primary ovarian tumor was between 33 and 84 years (average age, 52.23 years; median, 53 years). None of these women had a medical history of surgical operation for neoplastic disease. The 14 patients underwent surgical treatment of abdominal hysterectomy, bilateral salpingo-oophorectomy, appendectomy, and omentectomy with careful examination of all serosal surface and biopsies of any suspected lesions. All patients were staged according to the modified staging system of the International Federation of Gynecology and Obstetrics for malignant surface epithelial-stromal tumors. Twelve cases were FIGO stage I, and 2 cases were stage III.[14] Twelve of the 14 cases of primary ovarian carcinomas were unilateral with a size range of 6 to 15 cm, whereas 2 cases had bilateral masses with peritoneal metastasis outside the pelvis. On these 14 patients, a postoperative chemotherapeutic treatment was performed independently of the presence or absence of residual disease. The follow-up period was 26 to 143 months.

The age range of the patients with ovarian metastases was between 33 and 86 years (average age, 61.31 years; median, 67 years). In 3 of these patients (13.63%), secondary ovarian neoplasias were metachronous in regard to colon carcinoma. They had undergone surgical treatment of colectomy with regional lymphadenectomy and postoperative chemotherapeutic treatment of a primary intestinal carcinoma 18, 22, and 30 months previously. Soon afterward, these patients underwent surgical treatment of abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy for an ovarian mass. In 25 patients (81.81%) with secondary ovarian tumor, the ovarian metastases were synchronous with intestinal carcinoma. These 25 women had undergone surgical treatment of colectomy with regional lymphadenectomy, appendectomy, abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy, and subsequent chemotherapeutic treatment. In the remaining patient of our series of cases, the secondary ovarian tumor was anticipated in regard to the colon carcinoma. This woman had undergone abdominal hysterectomy, bilateral salpingo-oophorectomy, appendectomy, and omentectomy for an ovarian mass, and 23 months thereafter, she underwent a colectomy with regional lymphadenectomy for intestinal carcinoma.

Immunohistochemistry

The specimens were obtained by surgical resection in all cases and fixed in 10% formalin before being processed in paraffin. For immunohistochemical analysis, a representative section of each lesion was selected.

The immunohistochemical study was performed using the streptavidin-biotin-peroxidase method (UltraVision kit; Lab Vision, Fremont, CA) with diaminobenzidine as chromogen and hematoxylin as nuclear counterstain with antibodies anti–CDX-2 (clone 7C7/D4; Bio-Genex, San Ramon, CA; 1:100 dilution; with Immunostainer Genomix Bio-Genex and MW antigen retrieval for 30′ pH 6 with citrate buffer), anticytokeratin 20 (clone IT-Ks20.8; Bio-Genex; 1:60 dilution; with Immunostainer Nexes Ventana and protease digestion with phosphate buffer solution), anticytokeratin 7 (clone OV-TL12/30; Bio-Genex; 1:800 dilution; with Immunostainer Nexes Ventana and proteasi antigen retrieval with phosphate buffer solution), anti-CA125 (clone M11; CISbio International France; with Immunostainer Nexes Ventana), anti-CA19.9 (clone NS19.9; CISbio International France; with Immunostainer Nexes Ventana), and anticarcinoembryonic antigen (cd66e; clone 12-140-10; Novocastra, UK; 1:500 dilution; with Immunostainer Nexes Ventana and proteasi antigen retrieval with phosphate buffer solution).

The negative controls were performed by omitting the primary antibodies and by substituting the primary antibodies with nonimmune mouse sera. Appropriate positive and negative controls were run simultaneously. Sections of strongly positive cytokeratin (CK) 7 columnar epithelium cervical, positive CK20 and carcinoembryonic antigen (CEA) colonic adenocarcinoma, positive CDX-2 intestinal epithelium, CA125-positive serous ovarian carcinoma, and CA19.9-positive pancreatic carcinoma were used as positive control tissues for CK7, CK20, CEA, CDX-2, CA125, and CA19.9, respectively.

The immunohistochemically stained sections were evaluated without previous knowledge of the clinical outcome of each patient.

Brown staining of antibody-specific CDX-2 of the nucleus was considered positive. Brown staining of cytoplasm

of antibody-specific CK20 and CK7 was considered positive. Lesions were considered immunoreactive with CEA if the cytoplasm of columnar epithelial cells showed immunoreactivity equal to glycocalyx staining in intensity. Lesions were considered immunoreactive with CA125 and CA19.9 if they showed staining of the cell membrane, especially on the apical site. The intensity of immunostaining was classified in 3 grades: no staining, weak staining, and strong staining.

Statistical Analysis

Statistical analysis was performed to evaluate the reliability of the antibodies to discriminate between primary ovarian carcinoma and ovarian metastases.

The statistical analysis was performed using the Fisher exact test. P value ≤ 0.05 was considered statistically significant.

RESULTS

CDX-2 was diffusely expressed in the nucleus of all cases of secondary (intestinal) ovarian tumor (100%; Fig. 1), whereas the majority (78.57%) of primary ovarian mucinous carcinomas were CDX-2–negative (Fig. 2), and CDX-2 was expressed focally in the nucleus of only 3 cases (21.42%; Fig. 3) of PMOCs ($P < 0.0005$). Of these 3 cases of CDX-2 focally positive PMOCs, 2 were positive for CK7, CK20, CEA, and CA19.9 and negative for CA125, and 1 case was negative for CK7, CK20, CEA, CA19.9, and CA125.

The expression of CEA antigen was identical for both types of tumors: it was expressed in both cytoplasm and on the cell membrane strongly in 28 cases of secondary ovarian tumors (96.55%) and 12 cases of PMOCs (85.71%; $P = 0.24$). CK7 and CK20 showed a cytoplasmic localization in carcinoma cells: CK7 showed positive staining in 13 cases of secondary ovarian tumors (44.82%) and in 13 cases of PMOCs (92.85%), whereas CK20 showed positive staining in 17 cases of secondary ovarian tumors (58.62%) and in 6 cases of PMOCs (42.85%; $P = 0.003$ and $P = 0.51$, respectively). CA125 and CA19.9 predominantly showed staining in the cell membrane, especially on the apical site. Occasionally, some expression was found in the cytoplasm. CA125 was expressed

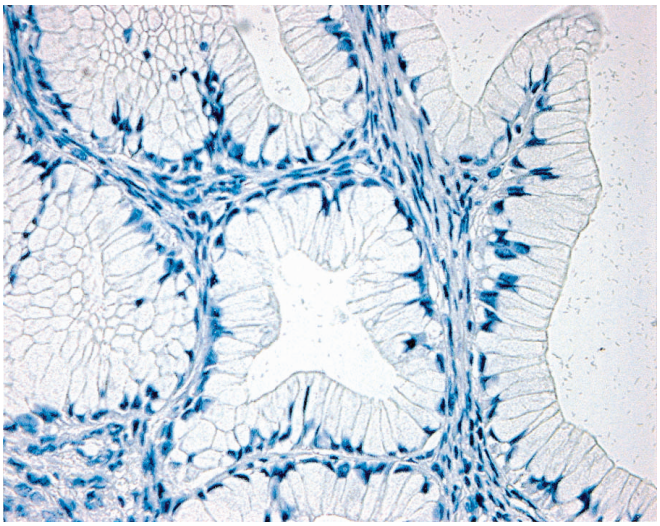

FIGURE 2. The majority (78.57%) of primary ovarian mucinous carcinomas were CDX-2–negative (original magnification ×40). Sections of secondary (intestinal) ovarian tumors and of primary ovarian carcinoma were run simultaneously.

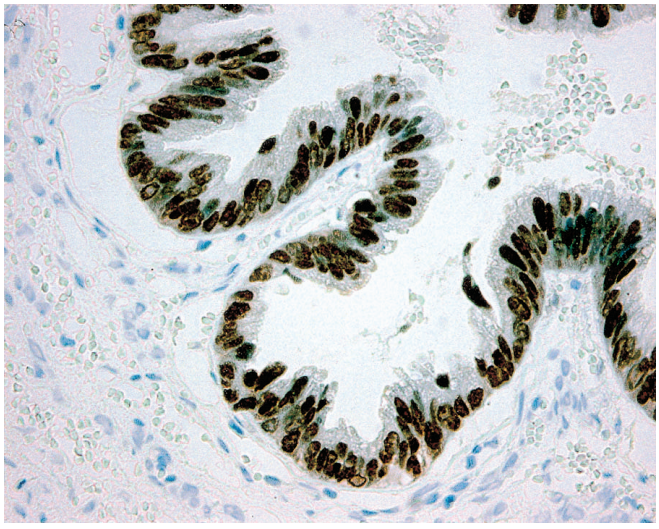

FIGURE 1. Metastasis of colorectal adenocarcinoma in the ovary. CDX-2 was diffusely expressed in the nucleus of the secondary (intestinal) ovarian tumor (original magnification ×40).

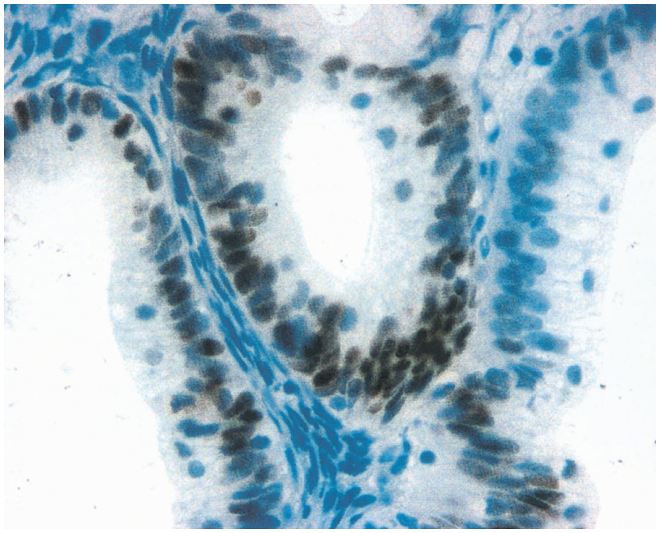

FIGURE 3. CDX-2 was expressed focally in the nucleus of only 3 (21.42%) cases of PMOCs (original magnification ×40).

in 3 cases of secondary ovarian tumors (10.34%) and in 4 cases of PMOCs (28.57%), and CA19.9 was expressed in all cases of secondary ovarian tumors (100%) and in 12 cases of PMOCs (85.71%; $P = 0.19$ and $P = 0.1$, respectively).

The results of the immunohistochemical studies are summarized in Table 1.

DISCUSSION

Often the diagnosis of a metastatic tumor to the ovaries is missed by the pathologist because the existence of a present or previous tumor in another side is either not known or disregarded.[15,16] Pathologists need to be extremely cautious when evaluating ovarian mucinous tumors, because secondary ovarian carcinomas of intestinal origin may closely simulate primary carcinomas of the ovary, and they may be much larger than intestinal primary tumor.[17]

The overall incidence of metastases of colorectal carcinoma to the ovaries is 10% when malignant ovarian tumors are encountered at the time of surgery.[18,19]

The histologic distinction between a secondary (intestinal) ovarian neoplasm and a primary ovarian mucinous adenocarcinoma can be extremely difficult and sometimes impossible: as many as 45% of secondary ovarian tumors are clinically seen to be primary ovarian carcinomas, and many are misinterpreted as such on pathologic examination, even when there is a known intestinal carcinoma.[5]

The difficulty of differential diagnosis on histologic grounds alone between primary ovarian carcinoma and colorectal metastases to ovaries has prompted an intensive search for immunohistochemical markers that can help distinguish between these 2 lesions.

Carcinoembryonic antigen is a highly glycosylated cell surface protein overexpressed in a variety of human tumors, such as colorectal, gastric, pancreatic, ovarian, breast, and nonsmall cell lung carcinomas.[20] It is characterized by an oncofetal pattern of developmental expression. Several studies have demonstrated that CEA immunostaining is of no value in the differentiation between secondary ovarian tumors showing a mucinous pattern and PMOC, because both show equally strong staining.[5,21]

CA125 and CA19.9 are not discriminatory markers between ovarian mucinous carcinoma and intestinal carcinoma.[21–23]

Cytokeratin 7 and CK20 have been suggested as immunohistochemical discriminators between ovarian masses of müllerian type (CK7+, CK20–) and of intestinal type (CK7–, CK20+), but whereas the discrimination of the nonmucinous carcinomas does not raise any problems, the distinction between a mucinous colonic and a mucinous ovarian carcinoma is more difficult. A few mucinous ovarian tumors resemble intestinal carcinomas in their immunohistochemical staining pattern (CEA+, CK20+, CK7–), and some colonic carcinomas expressed CK7.[21] A recent study reported that there are metastases from colorectal carcinomas with CK7+/CK20– immunophenotype and with CK7+/CK20+ immunophenotype, so these immunohistochemical stainings are not helpful for the differential diagnosis.[24] An important element in the evaluation of positive or negative staining is that focal expression of CK7 and CK20 should be given the same significance as negative immunostaining because of the steady increase of antibody sensitivity as a result of improved pretreatment methods.[25]

From these data, it was discovered that the specificity of CEA, CK20, CK7, CA125, and CA19.9 markers is not sufficiently high in distinguishing secondary (intestinal) ovarian tumors from primary ovarian mucinous carcinomas. The current study, which combines all 3—morphologic data, clinical data, and immunohistochemical assessment of malignant tumors of the ovary—was undertaken to determine whether the introduction of a new monoclonal antibody to an immunohistochemical panel, such as CDX-2, can help us in the differential diagnosis of mucinous ovarian carcinoma from the most frequent CDX-2–positive secondary (intestinal) ovarian lesions.[26]

In a recent study on metastases to the lungs, Barbareschi et al[24] analyzed 5 cases of secondary pulmonary mucinous tumors from ovarian neoplasia that were all CDX-2–positive, and Werling et al[27] found CDX-2 high-level expression in mucinous ovarian neoplasias that were also villin-positive.

On the other hand, our immunohistochemical data show that CDX-2 is a highly sensitive and specific marker to add in a limited immunohistochemical panel for distinguishing CDX-2–negative primary ovarian mucinous carcinomas from CDX-2–positive secondary (intestinal) ovarian tumors.

These immunohistochemical results may be of important clinical significance because most women with mucinous ovarian tumors have a hidden intestinal carcinoma or have al-

TABLE 1. Immunohistochemistry Analysis in Ovarian Mucinous Tumors

Tumor	CDX-2+ P/T (%)	CK7+ P/T (%)	CK20+ P/T (%)	CEA+ P/T (%)	CA125+ P/T (%)	CA19.9+ P/T (%)
Primary ovarian carcinomas	3/14 (21.42)	13/14 (92.85)	6/14 (42.85)	12/14 (85.71)	4/14 (28.57)	12/14 (85.71)
Secondary (intestinal) ovarian tumors	29/29 (100)	13/29 (44.82)	17/29 (58.62)	28/29 (96.55)	3/29 (10.34)	29/29 (100)

P/T, positive/total cases.

ready undergone surgical treatment of a previous colorectal tumor.[28]

The exact histologic diagnosis permits the best surgical and chemotherapeutic treatment of the individual patient. These results indicate the necessity, nevertheless, to investigate the CDX-2 marker on a larger series of mucinous ovarian lesions with known clinical history.

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