Primary Cervical Adenocarcinoma With Intestinal Differentiation and Colonic Carcinoma Metastatic to Cervix

An Investigation Using Cdx-2 and a Limited Immunohistochemical Panel

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● ***Context.*—Cdx-2 is expressed in normal colonic epithelia and in most colorectal adenocarcinomas. No data exist on Cdx-2 expression in primary cervical adenocarcinoma with colonic differentiation.**

***Objective.*—To ascertain the utility of Cdx-2 and a limited immunohistochemical panel in differentiating between primary cervical adenocarcinoma with intestinal differentiation and secondary (colonic) cervical adenocarcinoma, which call for different surgical and chemotherapeutic treatment protocols.**

***Design.*—We examined cervical tract adenocarcinomas in women with previously negative medical histories for neoplastic disease and in women with colonic carcinoma. An immunohistochemical panel consisting of cytokeratin 7, cytokeratin 20, carcinoembryonic antigen, and a new marker, Cdx-2, was evaluated in all cases. The clinical data, the morphologic features, and the immunohistochemical staining patterns were compared.**

***Results.*—Of the tumors diagnosed as metastatic intestinal adenocarcinoma of the cervix, based on clinical data and hematoxylin-eosin–stained sections, all were Cdx-2 positive, whereas Cdx-2 was not expressed in any of our cases of primary cervical adenocarcinoma with colonic differentiation. Carcinoembryonic antigen was expressed both in primary cervical tumor and in secondary (intestinal) cervical adenocarcinoma. Cytokeratin 20 was not expressed in our cases of cervical adenocarcinoma, and it was not expressed in 7.15% of cervical metastases from intestinal carcinoma. Immunostaining with cytokeratin 7 was positive in cervical adenocarcinoma, but was negative in secondary (intestinal) cervical adenocarcinoma.**

***Conclusions.*—Our immunohistochemical analysis shows that Cdx-2 has good specificity and would be a good marker to use in a limited panel of immunohistochemical markers, such as cytokeratin 7, cytokeratin 20, and carcinoembryonic antigen, to distinguish primary cervical adenocarcinoma from intestinal metastases to the cervix.**

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The incidence of adenocarcinoma of the cervix is variously reported as accounting for 7% to 22% of cervical malignancies.[1] Adenocarcinoma of the cervix shows a wide spectrum of glandular differentiation and is histologically subtyped as mucinous, endometrioid, clear cell, minimal deviation, well-differentiated villoglandular, serous, and mesonephric. The mucinous type encompasses several subgroups, including the most frequent, endocervical adenocarcinomas, which constitute approximately 90% of cervical adenocarcinomas,[2] as well as rare cases of signet ring cell and intestinal cell types.[3]

Primary adenocarcinoma of the cervix with colonic differentiation is very uncommon, and it is histologically indistinguishable from secondary (intestinal) cervical adenocarcinoma.[4–6] Certain features, however, including the location of the tumor, its growth pattern, and clinical history, have been suggested to be useful in distinguishing secondary cervical tumors from primary cervical tumors.

While the impact of the histologic subtype on survival is still a matter of dispute,[7,8] the distinction between a primary neoplasm and a metastasis to the endocervix is eminently important for treatment and prognosis. Establishing the histologic diagnosis of a tumor with an exclusive intestinal morphology as primary or metastatic cervical neoplasia on cervical biopsy may not always be possible with routine light microscopy alone. For the clinician, this differential diagnosis is essential in setting up different treatment strategies. The medical history of the patient and clinical workup with pelvic examination, measurement of tumor markers (CA 125, carcinoembryonic antigen [CEA], and CA 19.9), pelvic and abdominal ultrasonography, and computed tomography of the pelvis and the abdomen can lead pathologists to the correct diagnosis in most cases.

Homeobox genes of the caudal family, *CDX1* and *CDX2*, are expressed in the intestinal epithelium.[9] They are necessary for intestinal organogenesis and encode for nuclear transcription factors involved in proliferation and differentiation of intestinal epithelial cells in fetal as well as adult tissue.[10] A recent study proposed a possible link be-

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tween Cdx-2 expression and colonic tumorigenesis.[11] Cdx-2 seems to be expressed in normal colonic epithelia and most colorectal adenocarcinomas.[12,13]

The present study is based on 105 cases of cervical adenocarcinoma; of these, 2 (1.9%) were primary cervical tumors with colonic differentiation. We also analyzed 2605 cases involving women with intestinal carcinoma; in 14 (0.53%) of these cases, the cervical wall was infiltrated by an intestinal carcinoma. We evaluated the usefulness of a limited immunohistochemical panel consisting of cytokeratin 7 (CK7), cytokeratin 20 (CK20), CEA, and a new marker, Cdx-2, for distinguishing between primary adenocarcinoma with intestinal differentiation and cervical metastasis of colonic adenocarcinoma.

MATERIALS AND METHODS

The files of the Department of Human Pathology and Oncology of the University of Florence (Florence, Italy) were searched for the period 1980 to 2002 for the diagnosis of primary cervical adenocarcinoma. Of the 105 cases identified, we selected 2 cases (1.9%) of mucinous adenocarcinoma with intestinal differentiation. These 2 patients underwent cervical biopsy and subsequently abdominal hysterectomy with bilateral salpingo-oophorectomy and pelvic lymphadenectomy.

For the same period, department records contained surgical specimens from 7139 patients with intestinal adenocarcinoma. Of these 7139 cases, 2605 (36.48%) occurred in female patients, and in 14 cases (0.53%), the patients underwent surgical treatment consisting of colectomy with regional lymphadenectomy, appendectomy, abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy for intestinal tumor with uterine infiltration.

Clinical data available for each of the 14 patients with intestinal carcinoma infiltrating the cervix and for 2 patients with primary cervical adenocarcinoma included age, previous medical history, surgical treatment, stage of disease, and follow-up information.

We derived staging information from surgical notes and pathology reports. All neoplasms were staged according to a modified staging system of the International Federation of Gynecology and Obstetrics (FIGO).[14]

The specimens were obtained by surgical resection in all cases and were fixed in 10% formalin before being processed in paraffin. Hematoxylin-eosin–stained sections from each histologic specimen were reviewed by 2 pathologists to confirm the histologic diagnosis. For the immunohistochemical analysis, a representative section from each lesion was selected.

Immunohistochemical studies were performed using the streptavidin-biotin-peroxidase method (UltraVision kit, Lab Vision, Fremont, Calif) with diaminobenzidine as the chromogen and hematoxylin as the nuclear counterstain. Antibodies included anti-Cdx-2 (clone 7C7/D4; BioGenex, San Ramon, Calif; 1:100 dilution; with Immunocoloratore Genomix BioGenex and MW antigen retrieval), anti-CK20 (clone IT-Ks20.8; BioGenex; 1:60 dilution; with Immunocoloratore Nexes Ventana and protease antigen retrieval), anti-CK7 (clone OV-TL12/30; BioGenex; 1:800 dilution; with Immunocoloratore Nexes Ventana and protease antigen retrieval), and anti-CEA (CD66e; clone 12-140-10; Novocastra, Newcastle upon Tyne, United Kingdom; 1:500 dilution; with Immunocoloratore Nexes Ventana and protease antigen retrieval).

The negative control was performed by substituting the primary antibody with nonimmune mouse serum. Cases of conventional mucinous cervical adenocarcinoma and intestinal carcinoma were used as positive controls for immunohistochemical stains. Appropriate positive and negative controls were run simultaneously. The immunohistochemically stained sections were evaluated without previous knowledge of the clinical outcome of each patient. Brown staining of the nucleus with antibody-specific Cdx-2 was considered positive. Brown staining of the cytoplasm with 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 glycocalyceal staining in intensity.

RESULTS

Cervical Metastases

Fourteen patients with colonic carcinoma and synchronous cervical metastasis were studied. At the time of diagnosis, the women ranged in age from 47 to 76 years (average, 62 years). Other than cervical and regional lymph node metastasis, 5 women presented with ovarian infiltration, and in 2 patients the liver was infiltrated as well. The cervical tract metastases were detected during the clinical workup of patients before surgery or during the operation through careful examination of abdominal and pelvic viscera. Histopathologic findings of the cervical wall showed infiltrating ab extrinseco adenocarcinoma with morphologic features indistinguishable from the primary colonic tumor.

Primary Cervical Tract Tumors

A 49-year-old woman presented with a history of menometrorrhagia. Colposcopic examination revealed that the cervical surface had been replaced by swollen nodosities with atypical vessels. Vaginal examination and computed tomography revealed that the lesion did not infiltrate the parametrium. Tissue obtained by a cervical biopsy showed a carcinoma with signet ring features. Based on the morphologic features, an evaluation for an extragenital tumor was performed prior to definitive treatment. Upper gastrointestinal and sigmoidoscopy studies performed before surgery were negative for tumors, and the patient's breasts were normal to palpation and by mammography. The woman underwent a radical hysterectomy with bilateral salpingo-oophorectomy and a bilateral pelvic lymphadenectomy. During the operation, no evidence of extragenital tumor was found. The endocervical canal was completely obliterated by a tan tumoral mass, which extended deep into the cervical wall. Histopathologic findings showed an infiltrating carcinoma. The tumor cells had vacuolated cytoplasm and small angulated nuclei, which were displaced to the periphery of the cell. A single large intracytoplasmic vacuole occupied the cytoplasm of most cells. A diagnosis of primary cervical adenocarcinoma of signet ring type was made on the basis of the clinical and histologic data. The tumor was classified as FIGO stage IIA. The patient received postoperative pelvic irradiation.

A 23-year-old woman presented with a history of postcoital vaginal bleeding and menometrorrhagia. Colposcopic examination revealed that the entire cervical surface had been replaced by a wide and ulcerative lesion, and computed tomography revealed that the lesion infiltrated the parametrium. Tissue obtained by a fractional curettage showed a well-differentiated mucinous adenocarcinoma that was composed of intestinal-type cells. Also in this case, the histologic features of the cervical tumor necessitated that an evaluation for the possible presence of an extragenital tumor be performed before definitive treatment. Upper gastrointestinal and sigmoidoscopy studies performed before surgery were negative for tumor. The diagnosis of primary cervical adenocarcinoma of intestinal type was made on the basis of the clinical and histologic data. The tumor was classified as FIGO stage III. The patient underwent preoperative chemotherapy

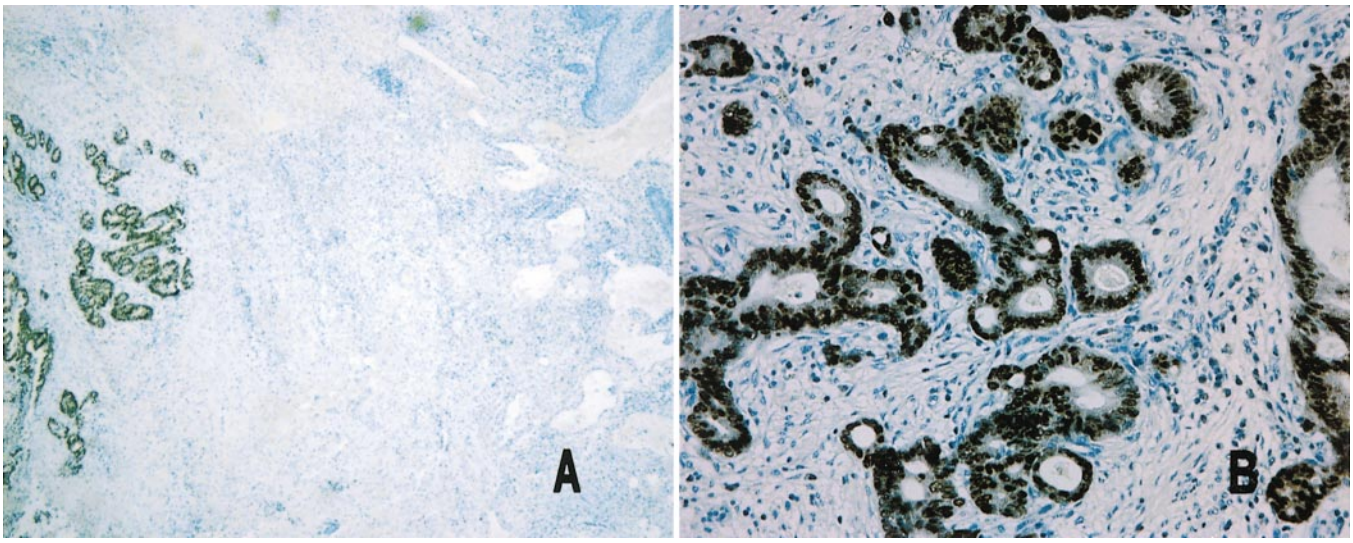

Figure 1. *Positive brown staining with antibody-specific anti–Cdx-2 in the cell nuclei of a colorectal adenocarcinoma metastasis in the cervical wall. A, Low-power view shows a glandular tumor with diffuse positive Cdx-2 staining infiltrating the cervix, while the squamous and glandular cervical epithelium are Cdx-2 negative (original magnification ×5). B, High-power view shows intensely positive Cdx-2 staining in the cell nuclei of intestinal carcinoma (original magnification ×40).*

and subsequent radical hysterectomy and bilateral pelvic lymphadenectomy.

Immunohistochemistry

Of the 14 tumors diagnosed as metastatic intestinal adenocarcinoma of the cervix (based on clinical data and hematoxylin-eosin–stained sections), all were positive for Cdx-2 (Figure 1) and CEA, and all were negative for CK7. Immunostaining with CK20 was positive in 13 cases (92.85%), but negative in 1 case (7.15%). The primary intestinal adenocarcinoma had the same pattern of immunohistochemical staining. Both intestinal and signet ring–type endocervical primary adenocarcinomas were positive for CK7 and CEA, but reactions for Cdx-2 and CK20 were completely negative (Figures 2 and 3, respectively) (Table).

COMMENT

The most common sites of metastatic involvement of colorectal carcinoma are regional lymph nodes and liver. Other relatively common metastatic sites include peritoneum, lung, and ovaries.[15] Apart from local pelvic tumor extension, metastatic cancer to the cervix is extremely rare, as shown in our data, accounting for 0.3% of all patients who die of cancer.[16]

Colonic differentiation features of carcinoma of the cervix are most commonly seen in metastatic lesions from the breast, large bowel, bladder, and stomach,[17–21] whereas primary endocervical adenocarcinoma of intestinal[22–25] or signet ring types[26–28] are very rare.

Symptoms relating to a primary carcinoma may remain clinically silent until months or years after the presentation of metastatic disease, and diagnosis of primary tumors with unusual morphologic features should be made only after exclusion of the most frequent metastatic tumors. Often the diagnosis of a metastatic tumor is missed by the pathologist because the existence of a present or prior tumor at another site is either not known or, if known, disregarded.

Pathologists need to be extremely cautious when evaluating cervical tumors with intestinal differentiation, because determination of whether a tumor is primary or metastatic has profound prognostic and therapeutic implications. Because there is considerable overlap in the morphologic features of primary and metastatic tumors, the importance of establishing a differential diagnosis based on histologic features has prompted a search for immunohistochemical stains that can help distinguish these 2 types of lesions. Carcinoembryonic antigen is a highly glycosylated cell surface protein that is overexpressed in a variety of human tumors, including cervical,[29] colorectal, gastric, pancreatic, ovarian, breast, and non–small cell lung carcinomas.[30]. Cytokeratin 7 and CK20 have been suggested as immunohistochemical markers of intestinal carcinoma (CK7 negative, CK20 positive), but some colonic carcinomas express CK7.[31] A recent study reported that there are metastases from colorectal carcinomas with a CK7-positive/CK20-negative immunophenotype and with a CK7-positive/CK20-positive immunophenotype, so this immunohistochemical staining is not helpful for differential diagnosis.[32]

This study, which combined both morphologic and clinical data and assessed the immunohistochemical properties of mucinous cervical adenocarcinomas with colonic differentiation, was undertaken with the aim of determining whether the introduction of a new monoclonal antibody, Cdx-2, in a panel of immunohistochemical stains can help in the differential diagnosis of cervical carcinoma with intestinal differentiation and the most frequent intestinal metastatic cervical lesions, which show Cdx-2 nuclear positivity.[33] At the moment, the literature contains no data on Cdx-2 expression in primary intestinal or signet ring cervical adenocarcinomas, and to our knowledge, this is the first report on Cdx-2 expression in primary adenocarcinoma of the cervix with colonic differentiation.

Our immunohistochemical data show that Cdx-2 is a good marker to use in a limited immunohistochemical panel to distinguish primary tumors from metastatic colonic lesions. These immunohistochemical data, when cor-

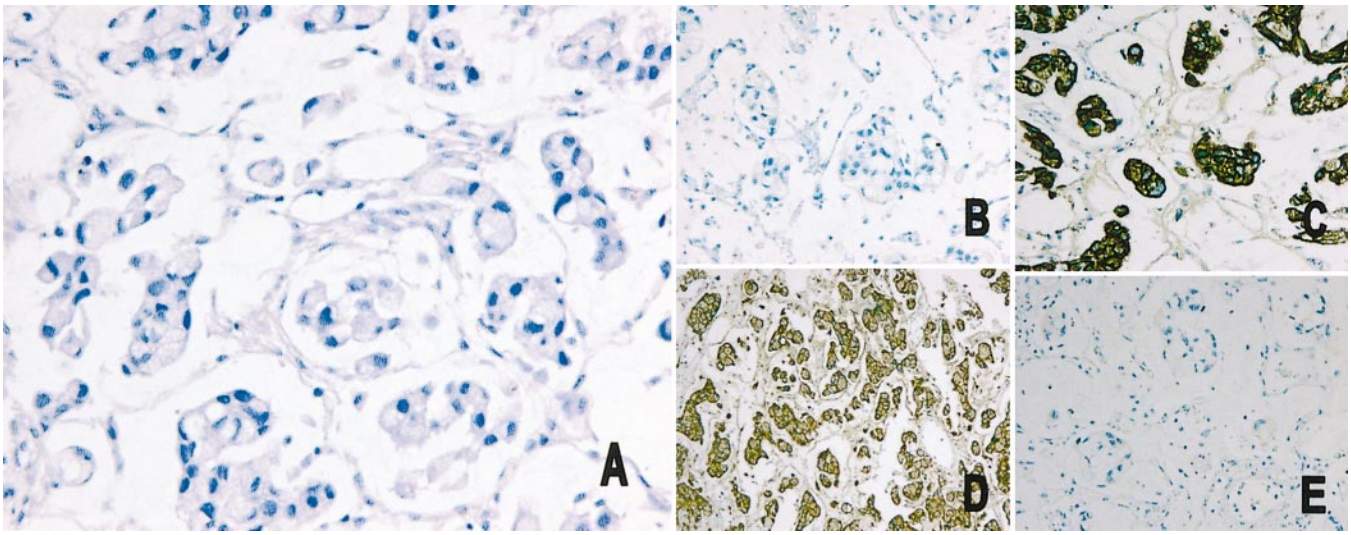

Figure 2. *A, Signet ring cell cervical carcinoma forming solid cell nests surrounded by pools of mucus (hematoxylin-eosin, original magnification ×40). B, Immunostaining with anti–Cdx-2 antibody shows negative reaction in signet ring cell nuclei (original magnification ×40). Immunostaining with anti–cytokeratin 7 antibody (C) (original magnification ×40) and with anti–carcinoembryonic antigen antibody (D) (original magnification ×40) shows positive signet ring cells. E, Immunostaining with anti–cytokeratin 20 antibody shows negative signet ring cells (original magnification ×40).*

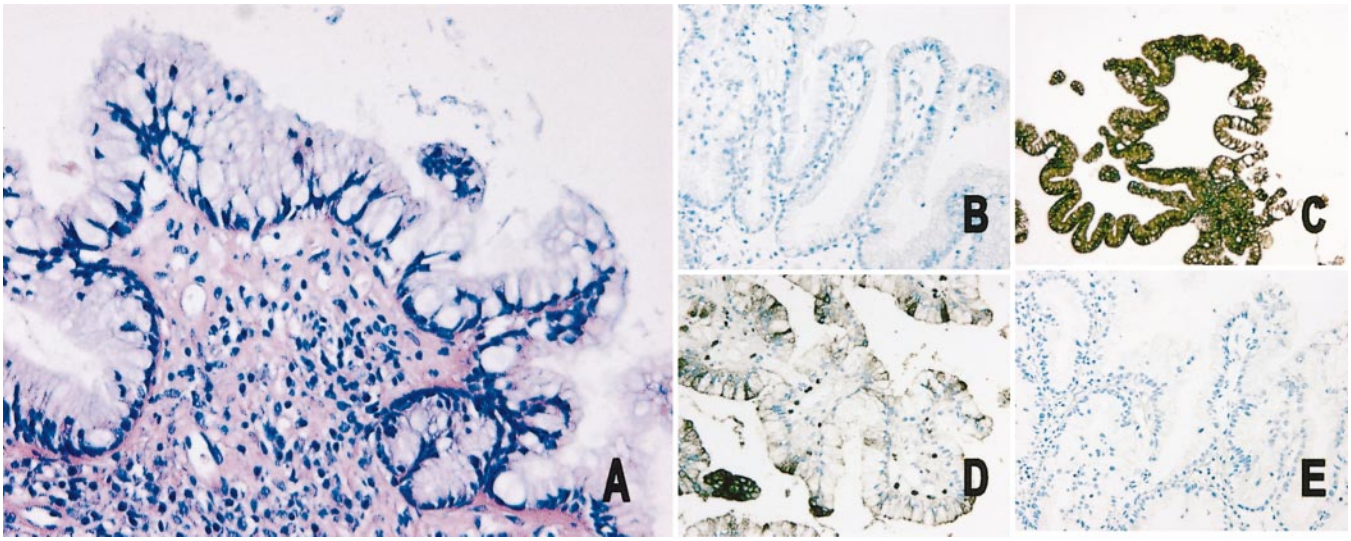

Figure 3. *A, Cervical adenocarcinoma with intestinal differentiation (hematoxylin-eosin, original magnification ×40). B, Immunostaining with anti–Cdx-2 antibody shows negative cell nuclei (original magnification ×40). Immunostaining with anti–cytokeratin 7 antibody (C) (original magnification ×40) and with anti–carcinoembryonic antigen antibody (D) (original magnification ×40) shows positive cells. E, Immunostaining with anti–cytokeratin 20 antibody (E) (original magnification ×40) shows negative cells.*

Immunohistochemistry Results				
Tumor Type	Cdx-2, No. of Cases Positive	Cytokeratin 7, No. of Cases Positive	Cytokeratin 20, No. of Cases Positive	Carcinoembryonic Antigen, No. of Cases Positive
Metastatic colonic adenocarcinoma (n = 14 cases)	14	0	13	14
Primary cervical adenocarcinoma with colonic differentiation (n = 2 cases)	0	2	0	2
Primary cervical adenocarcinoma (n = 103 cases)	0	103	0	103

related with clinical history and comparison of morphology, are important in establishing the correct diagnosis, which entails different therapeutic approaches. Our results also indicate the necessity of investigating the Cdx-2 marker on other primary cervical adenocarcinomas with intestinal differentiation.

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