Expression of the Intestinal Marker Cdx2 in Secondary Adenocarcinomas of the Colorectum

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• ***Context.*—Secondary adenocarcinomas of the large bowel can closely mimic primary tumors. The differentiation of secondary from primary adenocarcinomas of the colorectum, however, is important because their clinical management and prognosis are different. Immunostaining with the nuclear transcription factor Cdx2, expressed in normal intestinal epithelia and colorectal adenocarcinomas, could be of potential diagnostic use.**

***Objective.*—To investigate the diagnostic value of Cdx2 immunoexpression in distinguishing primary from common forms of secondary colorectal adenocarcinomas.**

***Design.*—Cdx2 immunoexpression was analyzed in 20 primary colorectal adenocarcinomas and in 34 secondary colorectal adenocarcinomas and their corresponding primary tumors. All secondary tumors were diagnosed through endoscopic biopsies and included 8 cases of ovarian (4 serous, 2 mucinous, and 2 endometrioid), 6 of mammary (4 lobular and 2 ductal), 4 of gastric (2 intestinal and 2 diffuse), 4 of pulmonary, 4 of pancreatic (ductal), 3 of prostatic, 3 of colorectal, and 2 of endometrial origin.**

***Results.*—Cdx2 was expressed in normal colorectal epithelium, in primary colorectal adenocarcinomas (20/20 cases), in secondary adenocarcinomas of colorectal (3/3) and gastric (3/4) origin, and in metastatic ovarian mucinous adenocarcinomas (2/2). In contrast, no Cdx2 immunoreactivity was observed in secondary colorectal tumors of ovarian (serous and endometrioid), mammary, pancreatic, pulmonary, prostatic, and endometrial origin.**

***Conclusion.*—Cdx2 immunostaining may be useful in discriminating primary colorectal carcinomas from frequent types of secondary colorectal adenocarcinomas of nongastrointestinal origin. We suggest including Cdx2 in any antibody panel put together to distinguish between primary and secondary epithelial colorectal malignancies.**

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Secondary neoplasms or metastatic tumors of the large intestine are relatively rare when compared with primary colorectal adenocarcinomas. The routes by which they reach the colorectum include direct extension, intraperitoneal spread, and lymphohematogenous embolization. The most common sites of origin of secondary colorectal adenocarcinomas are the ovary, breast, prostate, lung, stomach, uterus, colon, and pancreas.[1–4] Melanoma is the most common secondary malignancy among the nonepithelial tumors.[1–4] Discriminating primary from secondary colorectal carcinomas may be difficult for clinicians and pathologists, but this differential diagnosis is of crucial importance for therapeutic and prognostic purposes. The distinction may be particularly challenging in small colonoscopic biopsies, in which morphologic features suggestive of a nonintestinal origin may be difficult to identify.

The Cdx2 gene is an intestinal transcription factor involved in the proliferation and differentiation of intestinal epithelial cells. Previous studies showed that Cdx2 is expressed in normal and neoplastic intestinal epithelial cells with a relatively high sensitivity and specificity and that it can be used as an immunohistochemical marker for neoplasms of intestinal origin.[5–9] To our knowledge, the reactivity of Cdx2 in adenocarcinomas metastatic to the large bowel has not been yet studied.

The goal of the present investigation was to evaluate the expression of Cdx2 in frequent types of secondary colorectal adenocarcinomas so that we could determine whether Cdx2 is useful in discriminating primary from secondary colorectal tumors.

MATERIALS AND METHODS

Cases

Thirty-four cases of previously characterized, endoscopically diagnosed, secondary colorectal adenocarcinomas and their corresponding primary tumors from the Pathology Departments of the Hillel Yaffe, Sourasky, Meir, and Hasharon Medical Centers were evaluated. Adenocarcinomas that metastasized to the colorectum via hematogenous or peritoneal spreading and those that secondarily invaded the colorectal wall from neighboring organs were regarded as secondary tumors. Cases were selected in which the diagnosis was well established on the basis of both the histologic features of the primary tumor and the history provided in the surgical pathology report. For comparison, 20 cases of primary colorectal carcinoma, including 9 cases showing poor differentiation, were also examined. The secondary adenocarcinomas included 8 cases of ovarian (4 serous, 2 mucinous, and 2 endometrioid), 6 of mammary (4 lobular and 2 ductal), 4 of gas-

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Cdx2 Expression in Secondary Colorectal Adenocarcinomas

Tumor Source	No. of Cdx2-Positive*/Total Cases
Ovary	2/8
Serous	0/4
Mucinous	2/2
Endometrioid	0/2
Breast	0/6
Lobular	0/4
Ductal	0/2
Stomach	3/4
Intestinal type	2/2
Diffuse type	1/2
Lungs	0/4
Pancreas	0/4
Prostate	0/3
Colorectum	3/3
Endometrium	0/2

* All positive cases showed diffuse (2+) staining.

tric (2 intestinal type and 2 diffuse type), 4 of pulmonary, 4 of pancreatic (ductal), 3 of prostatic, 3 of colorectal, and 2 of endometrial origin. The secondary carcinomas of colorectal origin included 1 moderately and 2 poorly differentiated lesions. All original slides were reviewed and formalin-fixed, paraffin-embedded blocks containing well-preserved tumor specimens were selected for Cdx2 immunostaining.

Immunostaining

For immunohistochemical evaluation, sections were deparaffinized and subjected to high-temperature antigen unmasking in Tris buffer, pH 9.5, using an electric pressure cooker set at 118° for 4 minutes. The binding of Cdx2 (clone Cdx2-88, dilution 1:25; BioGenex, San Ramon, Calif) was detected using a labeled streptavidin-biotin peroxidase complex method. The formed complexes were visualized with aminoethyl carbazole chromogen/substrate. Sections were then counterstained with hematoxylin, dehydrated, cleared, and permanently mounted. Appropriate positive and negative controls were performed on each stained batch. The extent of immunostaining was scored semiquantitatively according to the estimated percentage of positive tumor cells in secondary colorectal carcinomas as follows: 0, no staining; 1+, focal, 1% to 19% reactive cells; 2+, diffuse, more than 20% reactive cells.

RESULTS

Results of the study are summarized in the Table. Cdx2 was diffusely (2+) expressed in primary colorectal adenocarcinomas (20/20 cases), in secondary adenocarcinomas of colorectal (3/3) and gastric (2/2 intestinal type and 1/2 diffuse type) origin (Figures 1 and 2), and in metastatic ovarian mucinous adenocarcinomas (2/2). In contrast, no Cdx2 immunoreactivity was observed in secondary colorectal tumors of mammary, pancreatic, pulmonary, prostatic, and endometrial origin or in metastatic ovarian serous and endometrioid carcinomas (Figure 3).

COMMENT

In the present study, we investigated the immunohistochemical expression of Cdx2 in a series of secondary colorectal adenocarcinomas and found that Cdx2 may be useful in discriminating primary colorectal carcinomas from secondary tumors of mammary, pancreatic, pulmonary, prostatic, ovarian (serous and endometrioid), and endometrial origin.

The incidence of secondary neoplasm involvement of the large bowel varies greatly, depending on the type of primary tumor as well as on the method of study. In the autopsy study of Berge and Lundberg,[10] the most common sources of metastatic colorectal disease were the lungs (22% of cases) and the breast (16%). In another series reporting colonic metastases diagnosed by biopsies, the most frequent sites of origin were the ovary (27% of cases), stomach (11%), uterus (9%), breast (7%), prostate (7%), and lungs (6%).[1] Secondary neoplasms may reach the large bowel by various routes. Direct extension or peritoneal seeding is seen with gastric, colonic, uterine, and ovarian adenocarcinomas. Prostatic and pancreatic malignancies can also infiltrate the colorectal wall by direct extension, whereas mammary and pulmonary tumors are the most frequent sources of hematogenous metastases.[1–4] The vast majority of gastrointestinal metastatic lesions from breast cancer are lobular.[11,12] Typically, they diffusely infiltrate the gastrointestinal wall instead of forming solitary masses, and they may mimic lymphomas. This is probably related to the loss of expression of the cell-cell adhesion molecule E-cadherin in the infiltrating lobular carcinoma.[13]

Colorectal metastases need to be differentiated from a primary colonic cancer. Misdiagnosing a potentially resectable primary colorectal carcinoma as metastatic or treating a metastatic lesion as primary colorectal cancer will result in inappropriate management. Grossly metastatic lesions can form polyps that are indistinguishable from ordinary mucosal colonic polyps. In other instances, they infiltrate the colonic wall, producing annular lesions mimicking primary colonic carcinoma, or they infiltrate diffusely, simulating a linitis plastica appearance.[1–4] Histologically, the presence of an intact colonic mucosa overlying a submucosal malignancy is suggestive of a secondary tumor. This finding in a colorectal biopsy, however, may also represent a submucosal extension of an adjacent primary carcinoma. On the other hand, metastatic tumors can invade the colorectal mucosa, thus mimicking primary malignancies. Immunohistochemical stains, such as cytokeratin 7 and cytokeratin 20, can be used in an attempt to assist in resolving this problem, but caution should be exercised when interpreting these immunostains, since they lack specificity, and overlapping immunohistochemical results have been reported.[14–16]

Human Cdx2 protein is a member of the homeobox gene that encodes an intestine-specific transcription factor. This protein regulates normal intestinal development and differentiation. It plays a critical role in triggering cells toward the phenotype of differentiated enterocytes as well as in maintaining the phenotype.[17–19] Cdx2 expression in adult nonneoplastic tissues is restricted to normal intestinal epithelium, a subset of normal pancreatic epithelial cells, and gastric and esophageal intestinal metaplasia.[6–9,20–23] In neoplasms, it was expressed by 86% to 100% of intestinal carcinomas[5–7,9] and less frequently by gastric adenocarcinomas, mainly intestinal-type tumors, and mucinous ovarian carcinomas.[5–7,9,20,21,24,25] In fact, our findings concurred with previous reports by showing that Cdx2 expression is limited to adenocarcinomas of colonic and gastric origin and ovarian mucinous carcinomas. As for Cdx2 expression in pancreatic ductal adenocarcinomas, there appears to be somewhat less agreement in the literature. Werling et al[7] reported scores of 2+ (26%–75% positive cells) and 3+ (>75% positive cells) positivity in 7 (32%)

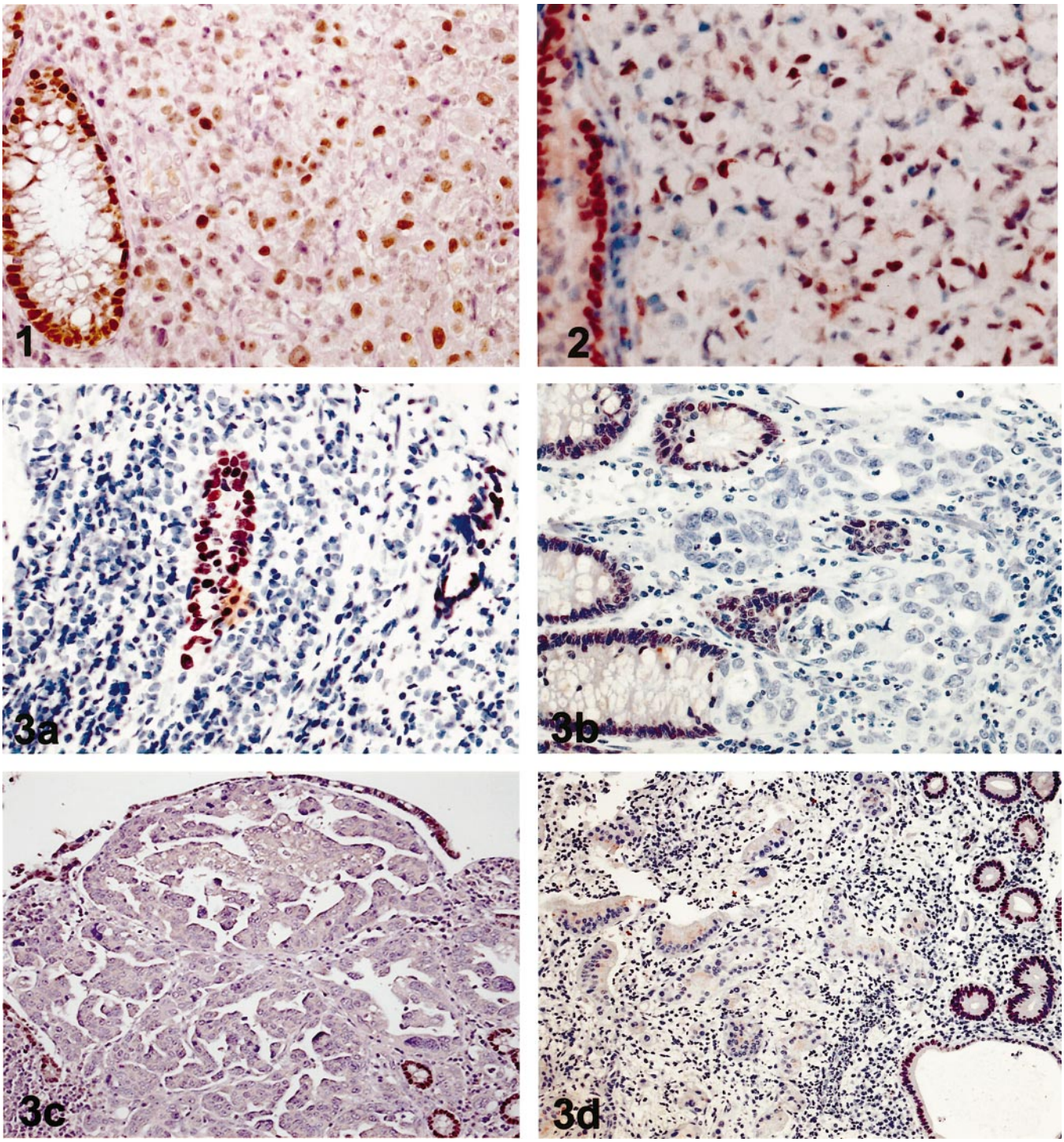

Figure 1. *Cdx2 is expressed by normal (left) and neoplastic colonic epithelium in a poorly differentiated colorectal carcinoma (original magnification ×200).*

Figure 2. *Cdx2 is positive in normal colonic epithelium (left) and metastatic poorly differentiated (diffuse type) gastric adenocarcinoma (original magnification ×200).*

Figure 3. *a, Colonic mucosa showing absence of Cdx2 immunoexpression in metastatic breast (lobular) carcinoma. Note positive reaction in residual normal colonic epithelium (original magnification ×200). b, Colonic mucosa showing negative Cdx2 immunostaining in metastatic pulmonary adenocarcinoma. Residual normal colonic epithelium is Cdx2 positive (original magnification ×200). c, Colonic mucosa showing absence of Cdx2 reactivity in secondary colorectal carcinoma of ovarian (serous) origin. Note positive reaction in residual normal colonic epithelium (original magnification ×100). d, Colonic mucosa showing negative Cdx2 immunostaining in secondary colorectal adenocarcinoma of pancreatic origin. Residual normal colonic epithelium is Cdx2 positive (original magnification ×100).*

of 22 cases, Moskaluk et al[6] found 1+ (<25% positive cells) expression in 8 (33%) of 24 cases, and in the series of Adsay et al,[26] Cdx2 reacted with 12 (16%) of 74 cases. In contrast, Kaimaktchiev et al[9] found only 2 (3%) of 70 cases positive for this marker. In our study, all 5 secondary tumors of pancreatic ductal origin failed to express Cdx2. Conflicting results have been also reported on Cdx2 immunostaining of poorly differentiated colorectal adenocarcinomas. Hinoi et al[27] demonstrated that a rare subset of poorly differentiated colonic carcinomas termed large cell minimally differentiated carcinoma or medullary carcinoma are characterized by microsatellite instability and loss of Cdx2 expression. Kaimaktchiev et al[9] recently studied tissue microarray samples of 1109 colorectal adenocarcinomas and found a lack of Cdx2 reactivity in 50 (6.7%) of 747 moderately differentiated tumors and in 14 (28%) of 50 poorly differentiated tumors. They concluded that Cdx2 expression decreases with tumor differentiation. Other series, however, failed to find a strong correlation between Cdx2 expression and the level of differentiation in colorectal adenocarcinomas. In the study of Werling et al,[7] 74 of 75 colonic carcinomas showed high levels of Cdx2 expression (2+ or 3+). Although several high-grade tumors showed scores of 2+ (26%–75% positive cells) compared with scores of 3+ (>75% positive cells) that were observed in all well-differentiated carcinomas, the authors concluded that the expression of Cdx2 did not appear to correlate with the level of tumor differentiation. Moskaluk et al[6] analyzed 60 microarray tissue samples of colorectal adenocarcinoma, including a nonspecified number of poorly differentiated tumors, and Cdx2 was positive in all cases. In the study of Barbareschi et al,[5] 58 of 60 colonic carcinomas expressed Cdx2 with a score of 3+ (51%–100% positive cells). The only 2 negative cases had microsatellite instability and were characterized by poorly differentiated morphology, confirming that the loss of Cdx2 expression may be present in a rare subgroup of poorly differentiated tumors with microsatellite instability.[5,27] In our study, all cases of poorly differentiated colorectal adenocarcinomas (9 primary and 2 secondary) were Cdx2 positive.

The potential utility of Cdx2 immunoexpression in the diagnosis of secondary colorectal adenocarcinomas was corroborated by our finding that secondary adenocarcinomas that arose outside the gastrointestinal tract were Cdx2 negative, with the exception of ovarian mucinous carcinomas. Cdx2 immunostaining may be particularly useful in cases of colorectal tumors developing in patients with a known primary adenocarcinoma arising in the breast, lungs, pancreas, prostate, endometrium, and ovaries (serous and endometrial types). Histopathologic comparison with the original cancer specimen and an appropriate panel of immunohistochemical stains including Cdx2 will facilitate the diagnosis of secondary cancer in the colorectum, thereby allowing the initiation of appropriate systemic oncologic therapy and avoiding unnecessary surgery.

In summary, Cdx2 is useful in discriminating primary from frequent forms of secondary colorectal adenocarcinomas of nongastrointestinal origin. As with any other marker, it should be interpreted only in the context of the overall clinicopathologic scenario and as a component of a panel of immunostains.

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