SATB2 Versus CDX2

A Battle Royale for Diagnostic Supremacy in Mucinous Tumors

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• ***Context.***—**Metastatic mucinous tumors present a diagnostic challenge for pathologists as tumor histomorphology is often nonspecific and optimal immunoprofiles are still under investigation.**

Objective.—**To present a head-to-head comparison of special AT-rich sequence-binding protein 2 (SATB2) and caudal type homeobox 2 (CDX2) expression in a diverse array of primary mucinous tumors.**

Design.—**SATB2 and CDX2 immunohistochemical stains were performed on whole sections from 44 mucinous colorectal carcinomas and 175 noncolorectal mucinous tumors. A nuclear scoring system measuring intensity (0–3+) and percentage staining (0 = <5%, 1 = 5%–49%, 2 = ≥50%) was implemented, producing an additive histologic score (H-score).**

Results.—**SATB2 demonstrated acceptable accuracy at low to moderate expression levels (H-scores of 1–4). With these H-score cutoffs, overall accuracy was greater than 90%. In contrast, CDX2's accuracy rivaled that of SATB2 only at an H-score of 5 (89.0%), as its specificity suffered at lower expression levels (<70.0% at H-scores of 1–4). Using a moderate H-score cutoff of 3 or higher, significant differences for both sensitivity and specificity were identified between SATB2 and CDX2 ($P = .01$ for sensitivity and $P < .001$ for specificity), though these stains were near equivalent when each was interpreted as positive at its respective optimal H-score (SATB2 ≥ 3 and CDX2 = 5).**

Conclusions.—**SATB2 is a more accurate marker of colorectal origin across a variety of expression levels compared with CDX2 when applied to mucinous tumors from a host of primary sites. However, these stains are near equivalent when each is interpreted at its optimal expression level.**

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Metastatic mucinous tumors pose a unique diagnostic challenge for the surgical pathologist as their morphologic and clinical characteristics are often nonspecific. The colorectum is the most common site of origin.[1,2] The World Health Organization[3] defines mucinous colorectal adenocarcinomas (mCRCs) as those containing greater than a 50% extracellular mucinous component. In addition, it is well known that these tumors may also arise as primaries from a variety of other sites, including the breast, lung, and upper gastrointestinal and gynecologic tracts.[1]

Historically, the immunophenotypic characteristics of mucinous tumors demonstrate a great degree of overlap across primary sites. Nonspecific staining characteristics of low-molecular weight-cytokeratins (CK7, CK20) have been well documented in mucinous tumors, including those of ovarian, colorectal, and pulmonary origin.[1,4–7] To compound this, caudal type homeobox 2 (CDX2), a transcription factor expressed within the nuclei of intestinal epithelial cells, although highly sensitive for adenocarcinomas of colorectal origin, has been shown to exhibit promiscuous staining in a variety of other primary mucinous tumors.[1,8–10] This has been most extensively studied in those of ovarian origin, where CDX2 expression has ranged from 26% to 79%.[1,5,11–12]

As the need for a more colorectal-specific marker has emerged, special AT-rich sequence-binding protein 2 (SATB2) has been used with success and promise.[13] As a marker of glandular epithelium of the lower gastrointestinal tract (including the appendix), SATB2 has been used as a marker of metastatic mCRCs, most specifically compared with ovarian mucinous neoplasms (OMNs), primarily in tissue microarray (TMA)–based studies, with favorable sensitivity/specificity pairings.[12,14–16] Despite this, few studies have examined the expression of SATB2 in mucinous tumors from a variety of other primary sites.[17] Our previous work addressed this issue with data on SATB2 expression specifically in mucinous neoplasms from the colorectum, breast, lung, ovary, uterus (including endocervix), pancreas, and upper gastrointestinal tract (stomach and esophagus).[18] As expected, we found reduced sensitivity of SATB2 as a marker of mCRC compared with conventional (nonmucinous) type (83% versus 98%).[18] However, SATB2 still performed well in distinguishing mCRC from mucinous tumors of noncolorectal origin, with a specificity of 95%.[18]

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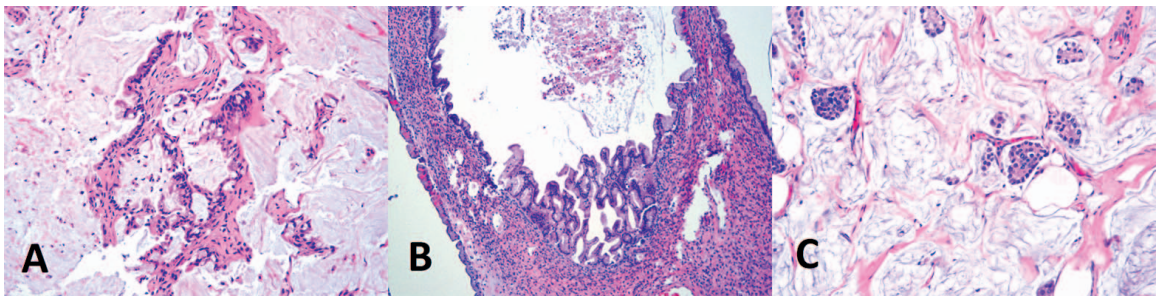

Figure 1. *Examples of mucinous tumors in our study. A, Mucinous colorectal adenocarcinoma with strips of low-grade neoplastic epithelium floating in mucin pools. B, Mucinous borderline tumor of the ovary. C, Mucinous adenocarcinoma of the breast displaying colloid features with epithelium of inverted polarity surrounded by large mucin pools (hematoxylin-eosin, original magnifications ×200 [A and C] and ×100 [B]).*

Few studies have directly compared CDX2 and SATB2 expression in mucinous tumors. Strickland et al[14] found that SATB2 was a more specific marker of lower gastrointestinal origin compared with CDX2. In this study,[14] SATB2 was expressed in 11.5% of upper gastrointestinal mucinous tumors (including gastric and pancreatobiliary) versus 50% for CDX2, and 1.7% of primary ovarian mucinous tumors versus 38.3% for CDX2. More recently, SATB2 staining has also proven to be an effective adjuvant marker in determining the primary site of metastatic Krukenberg tumors to the ovary compared with CDX2, including those with signet ring cell morphology.[19,20] However, the majority of the work in this area has dealt almost exclusively with the diagnostic challenge of mCRC in the form of appendiceal mucinous neoplasms versus primary OMNs. Sensitivity and specificity values in differentiating these entities have ranged from 83% to 94% and 78% to 98%, respectively, for SATB2 and 91% to 93% and 56% to 88%, respectively, for CDX2 in contemporary articles.[12,14,16,21]

Our research seeks to directly compare SATB2 and CDX2 expression in a wider array of primary mucinous tumors. Toward this effort, we use whole slide sections (in lieu of TMAs) to evaluate a greater degree of tumor expression and therefore provide better diagnostic understanding of their potential for diffuse versus focal staining. Additionally, we investigate whether there is any added value in using SATB2 versus CDX2 if they are each interpreted as markers of colorectal origin at their optimal expression levels.

MATERIALS AND METHODS

After institutional review board approval from the University of New Mexico (Albuquerque), an electronic archive search was performed to include all primary mucinous epithelial tumors diagnosed at our institution between the years 2001 and 2014. The following sites were included: colorectum, ovary, breast, lung, uterus (including cervix), pancreas, and stomach/esophagus. Additional inclusion criteria included the availability of paraffin blocks with adequate tissue for ancillary studies and hematoxylin-eosin–stained slides. After review of the hematoxylin-eosin–stained slides, tumors were designated as mucinous if the majority (>50%) of the neoplasm demonstrated a mucinous component (Figure 1, A through C). Tumors with less than a 50% mucinous component were excluded. Forty-four mucinous CRCs and 175 noncolorectal mucinous tumors were found in our archives. Of the noncolorectal mucinous tumors, 125 of 175 were mucinous adenocarcinomas and 50 of 175 were noninvasive mucinous tumors. All benign mucinous tumors were either ovarian cystadenomas/borderline cystadenomas or noninvasive pancreatic mucinous tumors (mucinous cystic neoplasms or intraductal papillary mucinous neoplasms).

These primary tumors were broken down by site of origin as follows: colorectal (44), ovary (60 total; 18 mucinous adenocarcinomas, 41 mucinous borderline tumors, 1 mucinous cystadenoma), breast (31), lung (26), uterus (28 total; 26 endometrial, 2 endocervical), pancreas (15 total; 7 mucinous/colloid adenocarcinomas, 6 mucinous cystic neoplasms, 2 intraductal papillary mucinous neoplasms), and stomach/esophagus (15). In regard to the technical aspects and stain-scoring approach of this study, our method mirrored that of the one described in our group's previous work on SATB2 staining in mucinous tumors.[18] To summarize, tumor blocks with the greatest amount of epithelium were selected for each case. SATB2 (Cell Marque 384R)-stained slides were generated for each tumor block via the following protocol: Blocks from formalin-fixed, paraffin-embedded tissue were sectioned at 4 to 5 μm and subsequently mounted on charged (+) slides. These slides were baked at 60°C for a period of 60 minutes. Before application of the individual SATB2 antibody, slides were treated with Discovery Cell Conditioner #1 (Ventana 950-500) for 36 minutes at 95°C. The Ventana Discovery platform was used in application of the SATB2 antibody. SATB2 (Cell Marque 384R) was diluted (1:25) in Discovery P.S.S. Diluent (Ventana 760-212), applied to individual slides, and incubated for 16 minutes at 37°C. This was followed by antibody detection via OmniMap anti-Rb HRP (Ventana 760-4311) and Discovery DAB CM (Ventana 760-159).

CDX2 (Abcam ab76541)-stained slides were generated for each tumor block via the following protocol: Blocks from formalin-fixed, paraffin-embedded tissue were sectioned at 4 to 5 μm and subsequently mounted on charged (+) slides. These slides were baked at 60°C for a period of 60 minutes. Before application of the individual CDX2 antibody, slides were treated with Discovery Cell Conditioner #1 (Ventana 950-500) for 8 minutes per application × 3 applications at 100°C. The Ventana Discovery platform was used in application of the CDX2 antibody. CDX2 (Abcam ab76541) was diluted (1:1000) in Discovery P.S.S. Diluent (Ventana 760-212), applied to individual slides, and incubated for 32 minutes at 37°C. This was followed by antibody detection via anti-Rb HQ and anti-HQ HRP (Ventana 760-4815 and 760-4820) and Discovery DAB CM (Ventana 760-4304). The slides were then counterstained with a hematoxylin (Ventana 760-2021) and bluing solution (Ventana 760-2037). They were then removed from the autostainer and coverslipped manually.

A previously validated scoring system[18] analogous to the Allred estrogen/progesterone receptor scoring system used in breast cancer was used. The nuclear intensity of SATB2/CDX2 staining was scored as negative, 0; weak, 1+; moderate, 2+; or strong, 3+ (Figure 2, A through C) relative to the intensity of the respective SATB2 and CDX2 controls of normal colon. Percentage of tumor staining was scored based on 3 separate categories: majority of tumor staining (≥50%) = 2, minority of tumor staining (5%–49%) = 1, and negative (<5%) = 0. These intensity and percentage scores were then added to generate a final histologic score (H-score), with a score of 0 representing cases with completely absent intensity/percentage staining and a score of 5 representing cases with maximal intensity/percentage staining (Figure 3, A and B). All preliminary scoring was performed by 2 pathologists. The

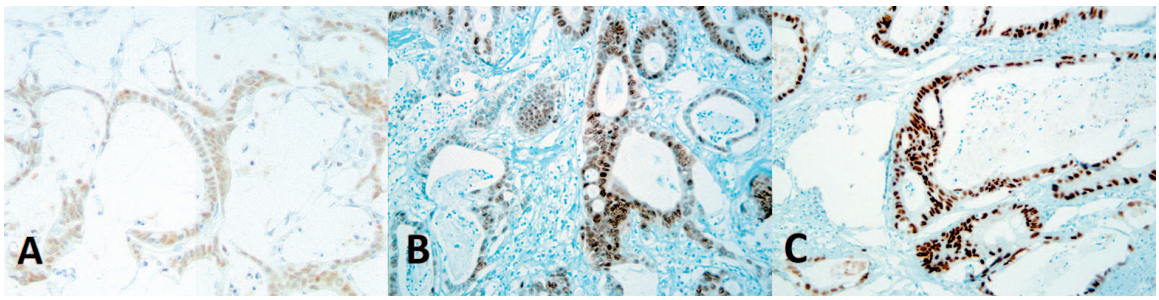

Figure 2. *Example of nuclear intensity score (comprising one component of the histologic score) in mucinous colorectal carcinomas. A, 1+ intensity. B, 2+ intensity. C, 3+ intensity (SATB2, original magnification ×200 [A]; CDX2, original magnification ×200 [B and C]).*

pathologists were blinded to the tumor site of origin. Each observer was blinded to the other's H-scores. Several cases showed intensity and/or percentage scores close to the defined cutoffs, and these cases were resolved by evaluating the slides at a double-headed microscope with the senior author of the study (J.A.H.).

The sensitivity, specificity, and accuracy percentages in differentiating mucinous tumors of colorectal origin from those from noncolorectal sites were calculated from binary contingency tables with exact binomial 95% CIs for each stain and H-score cutoff. Subsequently, sensitivity and specificity values were compared between selected stains using a McNemar test for paired comparisons with χ^2 and P values being calculated. All analyses were performed using MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016).

RESULTS

We evaluated the staining characteristics (intensity and percentage positivity) of each mucinous tumor from colorectal and noncolorectal sites as shown in Tables 1 and 2. Any SATB2 staining was observed in 39 of the 44 mCRC cases (88.6%) and any CDX2 positivity was observed in all of the mCRCs examined (44; 100%). Of the mucinous noncolorectal tumors (175), 13 (7.4%) demonstrated SATB2 positivity, whereas 96 (54.9%) showed CDX2 positivity.

The H-score (combining intensity and percentage scores) was derived to define a true-positive result (mucinous tumors of colorectal origin) in an attempt to provide maximum diagnostic accuracy. Accordingly, sensitivity and specificity values were calculated using binary contingency tables for each H-score (using various cutoff values: 1–5 total H-score). The sensitivity and specificity of various H-scores are provided in Table 3 (with 95% CIs), along with an overall accuracy percentage. SATB2 showed its greatest diagnostic value with an H-score of 3 or higher (maximal overall accuracy of 92.2%), meaning one should see at least either moderate- or high-intensity staining in a minority of tumor cells or weak staining in a majority of the tumor to infer colorectal origin. In contrast, CDX2 demonstrated its greatest overall accuracy (89.0%) with an H-score of 5, as its specificity values were drastically reduced at H-scores less than 5 (Table 3). Because a SATB2 H-score higher than 3 and a CDX2 H-score of 5 provided the greatest accuracy for each marker, we calculated positivity rates in all mucinous tumors for both markers at or above these optimal thresholds (Table 4). There were no significant differences in sensitivity or specificity when each stain was interpreted at its optimal H-score ($P = .75$ for sensitivity and $P = .17$ for specificity). However, if both stains were considered positive at the median H-score cutoff of 3 or higher, SATB2 significantly outperformed CDX2 in total accuracy and specificity ($P < .001$) but not in sensitivity ($P = .01$) (Table 3).

Of note, SATB2 and CDX2 each showed limited value in differentiating mucinous tumors of the colorectum (both 81.8% positive with optimal H-score cutoffs) from those of the upper gastrointestinal tract (sans pancreas), with 26.7% of the latter having SATB2 H-scores of 3 or higher and 33.3% having CDX2 H-scores of 5 (Table 4). Although the SATB2 false-positive rate in stomach/esophageal primaries was slightly favorable compared with CDX2 at their respective optimal H-score cutoffs, this was not statistically significant ($P > .99$).

Primary mucinous ovarian tumors showed false-positive STATB2 staining defined by an H-score of 3 or higher at a rate of 3.3%, whereas CDX2 marked these tumors at a rate of 10% at its optimal H-score of 5 (Table 4). Again, similar to the upper gastrointestinal primaries, this trend favored SATB2 in the ovarian primaries but was not statistically significant ($P = .22$).

SATB2 appeared to outperform CDX2 in mucinous pancreatic tumors, as it lacked expression in all of these neoplasms whereas CDX2 was positive (H-score = 5) in 26.7% (Table 4). However, this trend was not statistically

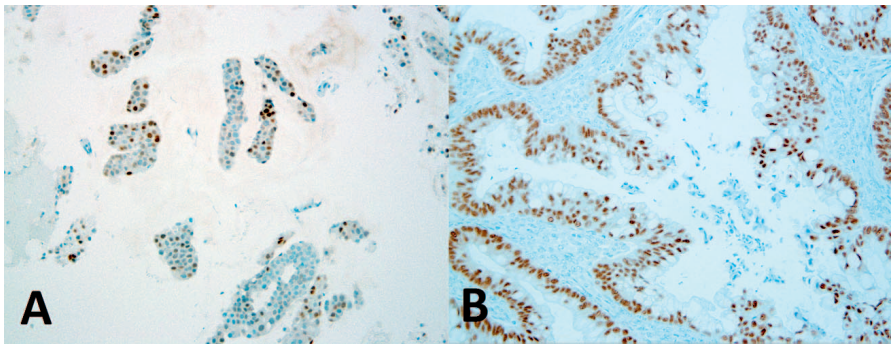

Figure 3. *Example of a mucinous breast adenocarcinoma with an SATB2 nuclear intensity score of 2+ with approximately 30% of the tumor staining. The resultant histologic score (H-score) was 3 and this was therefore considered aberrant positive staining (A). This mucinous adenocarcinoma of the ovary demonstrated diffuse SATB2 staining (>50%) at a nuclear intensity of 2+ for a resultant H-score of 4 (B) (original magnification ×200).*

Table 1. Any SATB2 Expression in Primary Mucinous Tumors

Score	Site, No. %						
	Colorectum (n = 44)	Ovary (n = 60)	Breast (n = 31)	Lung (n = 26)	Uterus (n = 28)	Pancreas (n = 15)	Stomach and Esophagus (n = 15)
Intensity							
1	8 (18.2)	1 (1.7)	2 (6.5)	0 (0)	1 (3.6)	0 (0)	1 (6.7)
2	18 (40.9)	2 (3.3)	3 (9.7)	0 (0)	0 (0)	0 (0)	3 (20.0)
3	13 (29.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
All positive	39 (88.6)	3 (5.0)	5 (16.1)	0 (0)	1 (3.6)	0 (0)	4 (26.7)
Percentage							
0	1 (2.3)	0 (0)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)
1	4 (9.1)	2 (3.3)	3 (9.7)	0 (0)	1 (3.6)	0 (0)	0 (0)
2	34 (77.3)	1 (1.7)	1 (3.2)	0 (0)	0 (0)	0 (0)	4 (26.7)

significant ($P = .13$), likely because of a lack of power, as the pancreatic cohort consisted of only 15 cases.

The only individual site where CDX2 generated fewer false-positive results than SATB2 was in differentiating colorectum from breast (9.7% SATB2-positive breast primaries versus 0% CDX2-positive breast primaries at H-scores of 3 or higher and 5, respectively; Table 4), but this was also not statistically significant ($P = .25$).

So that the reader can directly compare SATB2 and CDX2 positivity rates at each stain's optimal H-score cutoff, we have included Tables 5 (H-score ≥ 3 for both stains) and 6 (H-score = 5 for both stains), which indicate that CDX2's specificity suffers greatly if interpreted at the optimal SATB2 H-score of 3 or higher (Table 5) and SATB2's sensitivity is drastically reduced if interpreted at the optimal CDX2 H-score of 5 (Table 6).

DISCUSSION

Mucinous carcinomas of the lower gastrointestinal tract comprise approximately 1% to 6% of all colorectal adenocarcinomas.[22] Despite most commonly being associated with the colorectum, they can occur in a variety of other primary sites and resemble one another without specific histologic clues to their site of origin. En masse, these tumors are of an aggressive nature and are prone to metastases.[3] As treatment regimens differ based on a tumor's primary site of origin, it is critical for the pathologist to be able to assign a potential site of origin to these neoplasms. SATB2 and CDX2 have emerged as markers of the intestinal epithelium of the lower gastrointestinal tract. Most of the immunohistochemical expression data regarding these markers have been demonstrated in tumors of conventional (nonmucinous) histomorphology, whereas expression data in purely mucinous tumors are scarce.[10,17] We present the most extensive head-to-head comparison of SATB2 and CDX2 immunohistochemical staining to date using whole slide sections (in contrast to TMAs) of neoplasms from a variety of primary sites to fully characterize how these markers behave when tumors differentiate toward a mucinous phenotype.

We found that although CDX2 is a more sensitive marker of colorectal origin at a moderate expression level (H-score cutoff of ≥ 3 [sensitivity = 100.0%] compared with SATB2 [sensitivity = 81.8%]), it suffers from such weak specificity (57.7%) that its potential application in this setting is treacherous (Table 3). The ideal cut point for CDX2 would be at the highest expression level (H-score = 5), in which 89.0% of the tumors in our cohort were accurately identified. Interestingly, such high CDX2 expression (3+ nuclear intensity and >50% tumor staining) results in a sensitivity drop to 81.8%, equal to SATB2 at its ideal H-score of 3 or higher (Table 3), and its specificity nearly approaches that of SATB2 at SATB2's optimal H-score of 3 or higher. This indicates that these stains are nearly equivalent when applied to a large cohort of colorectal and noncolorectal mucinous tumors if they are interpreted

Table 2. Any CDX2 Expression in Primary Mucinous Tumors

Score	Site, No. %						
	Colorectum (n = 44)	Ovary (n = 60)	Breast (n = 31)	Lung (n = 26)	Uterus (n = 28)	Pancreas (n = 15)	Stomach and Esophagus (n = 15)
Intensity							
1	0 (0)	6 (10.0)	0 (0)	9 (34.6)	2 (7.1)	2 (13.3)	7 (46.7)
2	8 (18.2)	32 (53.3)	0 (0)	5 (19.2)	1 (3.6)	8 (53.3)	1 (6.7)
3	36 (81.8)	10 (16.7)	0 (0)	0 (0)	2 (7.1)	4 (26.7)	7 (46.7)
All positive	44 (100)	48 (80.0)	0 (0)	14 (53.8)	5 (17.9)	14 (93.3)	15 (100)
Percentage							
0	0 (0)	5 (8.3)	0 (0)	2 (7.7)	3 (10.7)	1 (6.7)	0 (0)
1	0 (0)	11 (18.3)	0 (0)	4 (15.4)	1 (3.6)	5 (33.3)	6 (40.0)
2	44 (100)	32 (53.3)	0 (0)	8 (30.8)	1 (3.6)	8 (53.3)	9 (60.0)

Table 3. Accuracy of SATB2 and CDX2 at Various Histologic Score (H-score) Cutoffs				
Stain	H-Score ≥	Sensitivity (95% CI)	Specificity (95% CI)	Overall % Accuracy (95% CI)
SATB2	1	88.6 (75.4–96.2)	92.6 (87.6–96.0)	91.8 (87.3–95.1)
CDX2	1	100 (92.0–100.0)	45.1 (37.6–52.8)	56.2 (49.3–62.8)
SATB2	2	86.4 (72.6–94.8)	93.1 (88.3–96.4)	91.8 (87.3–95.1)
CDX2	2	100 (92.0–100.0)	49.7 (42.1–57.4)	59.2 (53.0–66.4)
SATB2	3	81.8 (67.3–91.8)	94.9 (90.5–97.6)	92.2 (87.9–95.4)
CDX2	3	100 (92.0–100.0)	57.7 (50.0–65.1)	66.2 (59.5–72.4)
SATB2	4	68.2 (52.4–81.4)	97.1 (93.5–99.1)	91.3 (86.8–94.7)
CDX2	4	100 (92.0–100.0)	66.9 (59.4–73.8)	73.5 (67.2–79.2)
SATB2	5	27.3 (15.0–42.8)	100 (97.9–100.0)	85.4 (80.0–89.8)
CDX2	5	81.8 (67.3–91.8)	90.9 (85.6–94.7)	89.0 (84.1–92.9)

as positive only at their optimal expression levels. In comparison, SATB2 performed best in our cohort at an H-score of 3 or higher, where it correctly identified 92.2% of cases (Table 3), but suffered so severely in sensitivity at an H-score of 5 that one should not require strong *and* diffuse SATB2 staining to conclude that a mucinous tumor is of colorectal origin (Table 6). Therefore, in this head-to-head evaluation across all primary sites, it is clear that when expression levels are properly interpreted, SATB2 is not necessarily superior to CDX2 when inferring colorectal origin in a mucinous tumor of unknown primary.

Our findings are consistent with the existing data comparing SATB2 and CDX2 in site-specific mucinous neoplasms. We confirm the findings of previous studies directly comparing SATB2 and CDX2 expression in mCRCs (including appendiceal mucinous neoplasms) versus OMNs.[12,14,16,21] In head-to-head studies with comprehensive expression data comparing SATB2 and CDX2, SATB2 was expressed in 79% to 94% of mCRCs whereas CDX2 was expressed at a rate varying from 93% to 100.0%. To contrast, OMNs had a SATB2 expression rate varying from 2% to 22%, whereas CDX2 expression was found in 13% to 44% of these lesions.[12,14,21] Our positivity rates in mCRCs were generally consistent with these ranges; 88.6% and 100.0% of mCRCs showed any expression for SATB2 and CDX2, respectively (Tables 1 and 2) and SATB2 was positive in only 3.3% of OMNs at an H-score of 3 or higher (Table 4). In addition, only 5% of OMNs expressed any level of SATB2 (Table 1), supporting prior observations that SATB2 is an accurate marker in differentiating mCRCs from OMNs.[12,14,16,21] It also appears as if SATB2 may trend toward a lower false-positive rate compared with CDX2 in this setting even when both markers are interpreted at their optimal expression levels, as CDX2 was positive in 10% of OMNs at its optimal H-score of 5 compared with a 3.3% false-positive SATB2 rate at its optimal H-score of 3 or higher (Table 4), though this was not statistically significant in our OMN cohort ($P = .22$). In addition, the diagnostic value of CDX2 in OMNs suffers significantly at lower expression levels (70% CDX2-positive rate at an H-score ≥ 3; Table 5), indicating that one should require strong and diffuse CDX2 staining to infer colorectal origin if one is evaluating an OMN solely with this marker.

We also found that SATB2 and CDX2 were expressed to a moderate degree in our cohort of stomach/esophagus mucinous adenocarcinomas (26.7% at H-score of ≥ 3 for SATB2 and 33.3% at H-score = 5 for CDX2) and thus may be of more limited value in differentiating these from mCRC on a case-by-case basis when compared with mucinous tumors from most nongastrointestinal sites. Similar to what we observed in OMNs, CDX2 staining was so prevalent in these upper gastrointestinal primaries (100% showed any CDX2 expression; Table 2) that this marker is essentially useless in distinguishing mCRCs from their upper-tract counterparts if weak to moderate staining is interpreted as a positive result. SATB2, on the other hand, performs better in this setting at lower expression levels (26.7% positive rate at any expression level; Table 1), but does not appear to outperform CDX2 if one requires strong/diffuse CDX2 expression to infer colorectal origin (Table 4).

One area in which SATB2 may outperform CDX2 is in the setting of an mCRC versus a mucinous pancreatic tumor. Though our numbers were relatively low for the pancreatic category (15 tumors, 7 of which were invasive colloid

Table 4. Positive SATB2/CDX2 Expression in Mucinous Tumors Defined by an Optimal Histologic Score (H-Score) ≥ 3 for SATB2 and an Optimal H-Score = 5 for CDX2		
Primary Site of Mucinous Tumor (No.)	SATB2 Positive, No. (%)	CDX2 Positive, No. (%)
Colorectum (44)	36 (81.8)	36 (81.8)
Ovary (60)	2 (3.3)	6 (10.0)
Breast (31)	3 (9.7)	0 (0)
Lung (26)	0 (0.0)	0 (0)
Uterus (28)	0 (0.0)	1 (3.6)
Pancreas (15)	0 (0.0)	4 (26.7)
Stomach and esophagus (15)	4 (26.7)	5 (33.3)
Total noncolorectal mucinous tumors (175)	9 (5.1)	16 (9.1)

Table 5. Positive SATB2/CDX2 Expression in Mucinous Tumors Defined by a Histologic Score ≥ 3		
Primary Site of Mucinous Tumor (No.)	SATB2, No. (%)	CDX2, No. (%)
Colorectum (44)	36 (81.8)	44 (100.0)
Ovary (60)	2 (3.3)	42 (70.0)
Breast (31)	3 (9.7)	0 (0.0)
Lung (26)	0 (0.0)	8 (30.8)
Uterus (28)	0 (0.0)	2 (7.1)
Pancreas (15)	0 (0.0)	11 (73.3)
Stomach and esophagus (15)	4 (26.7)	11 (73.3)
Total noncolorectal mucinous tumors (175)	9 (5.1)	74 (42.3)

Table 6. Positive SATB2/CDX2 Expression in Mucinous Tumors Defined by a Histologic Score = 5		
Primary Site of Mucinous Tumor (No.)	SATB2, No. (%)	CDX2, No. (%)
Colorectum (44)	12 (27.23)	36 (81.8)
Ovary (60)	0 (0)	6 (10.0)
Breast (31)	0 (0)	0 (0)
Lung (26)	0 (0)	0 (0)
Uterus (28)	0 (0)	1 (3.6)
Pancreas (15)	0 (0)	4 (26.7)
Stomach and esophagus (15)	0 (0)	5 (33.3)
Total noncolorectal mucinous tumors (175)	0 (0)	16 (9.1)

carcinomas and 8 of which were in situ mucinous tumors), we observed no SATB2 staining whatsoever in these tumors (Table 1) compared with a 93.3% CDX2 positivity rate if any CDX2 expression was considered indicative of colorectal origin (Table 2). The CDX2 false-positive rate dropped to 26.7% (Table 4) at its optimal H-score of 5 but remained higher than the SATB2 false-positive rate of 0% (Table 4). Although this was not statistically significant ($P = .13$), we hypothesize that this is due to a power limitation in our cohort of only 15 pancreatic mucinous tumors. In fact, had the false-positive rates for SATB2 and CDX2 remained constant and our pancreatic cohort been doubled, we would have observed a statistically significant difference in expression (purported $P = .01$). Accordingly, we suggest this as a further avenue of study for research centers that see more of these particular tumors.

Interestingly, the lone primary site where CDX2 showed a better performance trend compared with SATB2 was in mucinous breast carcinomas. Few data are available on SATB2 expression in breast primaries. The seminal work on SATB2 immunohistochemical expression by Magnusson et al[17] notes that 6 of 147 presumably conventional breast cancers (4.1%) showed positive (albeit weak) nuclear staining for SATB2. Dragomir et al[22] evaluated 13 cases of breast adenocarcinomas with 2 demonstrating weak expression of SATB2 (15.0%). More recently, Yang et al[20] reported a small cohort of metastatic lobular breast adenocarcinomas to the ovary that demonstrated weak SATB2 positivity in 1 of 5 cases (20.0%). Of note, nuclear CDX2 expression was not identified in any of these same 5 cases.[20] Historically, nuclear CDX2 expression in breast primaries has been minimal to nonexistent (ranging from 0% to 2%).[23–31] In a study with 40 breast adenocarcinomas of exclusively mucinous phenotype undertaken by de Andrade Natal et al,[29] all cases were negative for CDX2 expression. Additionally, in a cohort of 33 breast and 50 gastrointestinal (upper and lower) signet ring cell adenocarcinomas reported by Hui et al,[30] CDX2 staining was 100% specific for tumors of gastrointestinal origin. Keeping in line with these data, our CDX2 false-positive rate was 0% in our purely mucinous cohort of breast adenocarcinomas regardless of H-score (Table 2) although any SATB2 expression was observed in 16.1% of cases. When controlled for optimal expression levels, our false-positive SABT2 rate in breast primaries decreased to 9.7% (Table 4), reducing its statistical significance compared with optimal CDX2 expression ($P = .25$). However, when our data are considered with the results generated from the aforementioned studies, this suggests at least a trend toward improved specificity for CDX2 compared with SATB2 in distinguishing mCRCs from mucinous breast adenocarcinomas.

The current study is limited by a lack of paired metastases to go along with the evaluated primary mucinous tumors. Although our previous work suggests that SATB2 staining is relatively preserved in metastatic tumor deposits, the corresponding correlate for CDX2 cannot be assumed.[18] Furthermore, expression data from several individual primary sites (including uterus, stomach/esophagus, and pancreas) were limited by relatively small cohorts. Although some would characterize our approach to defining a positive SATB2 result with an H-score of 3 or higher and positive CDX2 result with an H-score of 5 as overly restrictive, we would contend that this is a major advantage of our work. As SATB2's purported advantage over CDX2 is its increased specificity, we favored the use of a precise and rigid scoring system to evaluate our cohort. Given that pathologists are often unsure of how to interpret weak or focal immunohistochemical staining, we felt that a simple and reproducible scoring system could best address this potential pitfall with precise positivity rates across a myriad of expression patterns and resultant H-scores. Along this same vein, we prefer whole slide sections as opposed to TMAs to better reflect actual practice and to better understand the complete expression patterns that can be seen with these markers. As has been noted in our study and others prior, a significant percentage of noncolorectal lesions weakly express SATB2 in a low percentage of tumor cells, quantities that we would consider negative based on our preferred H-score cutoff of 3 or higher. Without evaluating the entire lesion on hand, scoring may lend itself to diagnostic overinterpretation or underinterpretation, calling into question the accuracy of TMA-based studies. As in the TMA-based evaluation of several immunohistochemical markers in appendiceal mucinous neoplasms and OMNs reported by Li et al,[12] positive staining was defined as 5% or more of tumor cells. A lack of quantitative intensity scoring of the nuclear stains in this study may have accounted for the somewhat elevated SATB2 positivity rate in OMNs (22.2%), as these lesions may have exhibited weak (1+) and focal (<50%) staining and therefore would not have met the positive threshold cutoff in our study or others similar.[12,14,16,21]

In conclusion, metastatic mucinous tumors present diagnostic challenges for surgical pathologists, as expression patterns of immunohistochemical stains have yet to be fully elucidated. Pathologists should be aware of the diagnostic advantages and potential challenges of one rival marker compared with another. We present SATB2 and CDX2 immunohistochemical expression data of primary site mucinous tumors using whole slide sections in a head-to-head evaluation, with full sensitivity and specificity pairings based on a simple quantitative scoring method, identifying an ideal cut point for assigning these lesions as colorectal origin depending on the stain evaluated (H-score $\geq$ 3 for SATB2, sensitivity = 81.8% specificity = 94.9%; H-score = 5 for CDX2, sensitivity 81.8%, specificity 90.9%). We surmise that if SATB2 and CDX2 are interpreted at proper expression levels, these stains are nearly equivalent in differentiating mCRCs from noncolorectal mucinous tumors as a whole. Unfortunately, both markers show significant expression in gastric and esophageal mucinous carcinomas, indicating that additional immunohistochemical stains should still be sought out that can more accurately differentiate these tumors from mCRCs. Interestingly, SATB2 may show higher specificity with regard to mucinous pancreatic tumors and

CDX2 may show higher specificity in mucinous breast carcinomas. We recommend this as a future avenue of study with larger cohorts to improve upon our admittedly limited data in these particular primary sites.

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