

The Role of SATB2 as a Diagnostic Marker for Tumors of Colorectal Origin

Results of a Pathology-Based Clinical Prospective Study

Anca Dragomir, MD, PhD,¹ Meike de Wit, PhD,² Christine Johansson,³ Mathias Uhlen, MD, PhD,⁴ and Fredrik Pontén, MD, PhD¹

From the ¹Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden; ²Department of Medical Oncology, VU Medical Center, Amsterdam, The Netherlands; ³Atlas Antibodies AB, AlbaNova University Center, Stockholm, Sweden; and ⁴Science for Life Laboratory, KTH-Royal Institute of Technology, Stockholm, Sweden.

Key Words: Immunohistochemistry; Cytokeratin 20; SATB2; Colorectal cancer; Cancer of unknown primary (CUP)

Am J Clin Pathol May 2014;141:630-638

DOI: 10.1309/AJCPWW2URZ9JKQU

ABSTRACT

Objectives: Immunohistochemistry is an important extension to clinical information and morphology, and prevails as an invaluable tool for establishing a correct cancer diagnosis in clinical diagnostic pathology. The applicability of immunohistochemistry is limited by the availability of validated cell- and cancer-type specific antibodies, rendering an unmet need to discover, test, and validate novel markers. The SATB2 protein is selectively expressed in glandular cells from the lower gastrointestinal tract and expression is retained in a large majority of primary and metastatic colorectal cancers.

Methods: We analyzed the expression of SATB2 in all clinical cases ($n = 840$), in which immunohistochemistry for detection of CK20 was deemed necessary for a final diagnosis.

Results: SATB2 showed a high sensitivity (93%) and specificity (77%) to determine a cancer of colorectal origin and in combination with CK7 and CK20, the specificity increased to 100%.

Conclusions: We conclude that SATB2 provides a new and advantageous supplement for clinical differential diagnostics.

Immunohistochemistry (IHC) is of vital importance to complement morphologic assessment of histochemically stained sections of tumor tissues in the routine diagnosis of cancer. With the availability of validated antibodies toward relevant targets, IHC can result in critical information on the type of tumor (diagnosis), grade of malignancy (prognosis), and susceptibility for specific anticancer drugs (treatment prediction). In cases in which patients first present with a metastasis or when patients present with tumors that are of morphologically ambiguous origin, eg, primary adenocarcinoma of the lung or a lung metastasis, it is important to determine a correct diagnosis to guide further investigations and to optimize therapeutic intervention.

Because of the intrinsic variation of tumor phenotypes, some of which present with dedifferentiated morphology, IHC prevails as an important tool to confirm the diagnosis also in cases in which clinical and morphologic information indicates a tumor of an almost certain histologic subtype and origin. With the exception of breast cancer, in which IHC for determining estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER-2) expression is part of the clinical diagnostic procedure, there is no systematic analysis of IHC-based expression patterns for the large majority of solid cancer types.

It is estimated that 3% to 5% of all cancers present as a metastasis from an unknown primary site, also known as cancer of unknown primary (CUP).¹ For these CUP cases and for the atypical cases in which clinical and morphologic data are not in agreement, IHC is necessary to narrow the range of diagnostic alternatives and, when possible, establish a tentative diagnosis with regard to cancer phenotype and most likely site of primary tumor. An increased repertoire

of cell and cancer type-specific antibodies would be of substantial benefit to optimize the diagnostic procedure when resolving differential diagnostic alternatives for CUP.

The success of immunopathology using IHC to develop algorithms for differential diagnosis depends on the availability of antibodies that target proteins selectively expressed in different defined cell and tumor types. Although the human genome encodes for approximately 20,000 protein-encoding genes,² only a small fraction of antibodies has been developed for use in clinical differential diagnostics. At present, validated antibodies detecting some 200 different proteins are used to determine the phenotype of tumors in the clinic. The vast majority of these antibodies recognize proteins expressed in several different cell types. With the exception of CD markers, which define various subtypes of hematopoietic and lymphoid cell types, cell type-specific protein expression patterns are rare.³ Examples of well-known antibodies targeting cell type-specific proteins include antibodies recognizing prostate-specific antigen (prostate glandular cells), thyroglobulin (thyroid glandular cells), melanA and tyrosinase (cells of melanocytic lineage), glial fibrillary acidic protein (astrocytic cells), and various hormones such as insulin, exclusively expressed in β cells in the pancreas.

At present there is no established specific marker for glandular cells of the lower gastrointestinal tract. Several protein expression patterns have been scrutinized and various markers have been tested in large series of different tumors. A handful of antibodies that provide useful information have been identified to confirm or reject a diagnosis of colorectal carcinoma (CRC). The most accepted antibodies for clinical differential diagnostics are antibodies detecting cytokeratin 20 (CK20). CK20 is an intermediate filament protein selectively expressed in glandular cells of the gastrointestinal (GI) tract, and is a highly sensitive marker. However, the specificity of CK20 alone is relatively low because this keratin is also expressed in the gastric epithelium, urothelium, and epidermal Merkel cells.⁴ In CRC, the expression of CK20 is generally preserved in metastases.⁵ Antibodies detecting caudal type homeobox 2 (CDX2) protein and cadherin 17 (CDH17) have also been used in the differential diagnostics of CRC because both these proteins are selectively expressed at relatively high levels in cells of the whole GI tract. In cases of unknown primary lesions, CDX2 has a relatively low predictive power because it is also expressed in gastric carcinomas (18%) and ovarian mucinous tumors (20%).⁶ CDH17 lacks in specificity as seen by the fact that CDH17 is also expressed in gastric, pancreatic, and biliary adenocarcinomas.⁷ In clinical practice, CK20 is often combined with other markers, such as the presence of CDX2 or the absence of cytokeratin 7 (CK7), to increase the accuracy for determining a diagnosis of CRC.

The special AT-rich sequence binding-protein (SATB2) is a DNA-binding protein, 733 amino acids long, which specifically binds to nuclear matrix attachment regions of DNA. SATB2 is involved in regulation of transcription and chromatin remodeling, and shows a remarkable degree of evolutionary conservation, with a difference of only three amino acids between mouse and human. In a recent study, antibodies detecting SATB2 were tested in more than 1,800 cases of CRC together with more than 600 cases of other tumors.⁸ The results suggested that IHC-based detection of SATB2 was highly specific for primary as well as metastatic lesions of CRC and showed a similar level of sensitivity for CRC as CK20, CDX2, and CDH17. Two recent studies suggest that downregulated expression of SATB2 is associated with metastasis and poor prognosis both in an animal model of CRC⁹ and in a prospective patient cohort.¹⁰ Other data support a role for SATB2 in head and neck squamous cell carcinoma¹¹ and ulcerative colitis.¹²

The promising findings of highly selective SATB2 expression in tumor cells from several retrospective cohorts of CRC and the combination of using IHC with antibodies detecting CK20 and SATB2 was reported to identify more than 95% of CRCs.⁸ This suggests that the concept should be tested in an unbiased clinical prospective study. In the present study, we analyzed the expression of SATB2 in more than 800 consecutive cases in which CK20 immunostaining was considered necessary for a final diagnosis. Our results suggest that immunostaining of SATB2 adds valuable information in clinical decision making to confirm or rule out tumors of colorectal origin.

Materials and Methods

Case Selection and IHC

The study was approved by the Department of Surgical Pathology, Uppsala University Hospital, Uppsala, Sweden, and all cases were prospectively selected from the clinical cases registered between June 2010 and December 2011. The inclusion criteria were defined as tissue specimens submitted to the department of pathology for diagnosis, where the pathologist in charge had performed an immunohistochemical staining of CK20 as part of the clinical diagnostic procedure. The total number of cases immunohistochemically stained for CK20 during this period was 840. The corresponding paraffin blocks from these cases were, in parallel with CK20, sectioned and immunostained for SATB2 expression. In accordance with current diagnostic guidelines, the pathologist in charge used several other antibodies in addition to CK20 (such as CK7, CDX2) and other ancillary methods to reach the final diagnosis. However, the

result of SATB2 staining was disregarded in the diagnostic process when cases were signed out by the pathologists.

IHC was performed after routine antigen retrieval using an automated IHC stainer (Autostainer, DakoCytomation, Glostrup, Denmark) as previously described.¹³ Primary antibodies against CK20 (mouse monoclonal M7019; DakoCytomation) and SATB2 (mouse monoclonal antibody AMAb90635, clone CL0276, Atlas Antibodies AB, Stockholm, Sweden) were used at a dilution of 1:200. All other antibodies deemed necessary for the diagnostic procedure were stained using the same instrumentation as specified before and at dilutions routinely established in the surgical pathology laboratory.

IHC Scoring

The CK20 and SATB2 immunohistochemically stained slides in all cases were reevaluated by two independent observers, one pathologist (A.D.) and one specially trained technician (C.J.). All disagreements were resolved by evaluating the slides with a double-headed microscope. The annotation process included a semiquantitative, categorical estimation of the fraction (%) of tumor cell nuclei positive for SATB2 regardless of intensity (nuclear fraction [NF]), which was scored 0 for less than 1%, 1 for 2% to 25%, 2 for 26% to 75%, and 3 for more than 75%.⁸ For the purpose of statistical analyses, SATB2 nuclear staining was further dichotomized into negative tumors (score 0) and positive tumors (scores 1-3). For CK20, a binary expression of the cytoplasmic staining was used, scored 1 for positive staining (any number of tumor cells) and 2 for negative staining, regardless of intensity. For the remaining immunohistochemical markers, the results were retrieved from the pathology records and were scored in a similar binary mode as for CK20. Representative images of SATB2 staining are illustrated in **Image 1**.

Establishing the Final Diagnosis

The final diagnosis was retrieved from the pathology record. No independent review of the diagnosis was attempted, because the authors had access only to a fraction of the diagnostic material, limited to the tissue sections selected for CK20 and SATB2 immunostaining. The final diagnosis was classified into one of four categories: "CRC," defined other non-CRC cancer ("other C"), "CUP," and benign tumor ("benign"). Information on whether the tumor was a primary cancer or a metastasis and the anatomic origin was recorded for cases in which such information was available. For the cases classified as CUP, the pathology database was assessed 6 months after the inclusion time, resulting in a follow-up time of 6 to 24 months. For cases in which new diagnostic data were available, the new diagnosis of the tumor type was recorded separately.

Statistics

Statistical analyses were carried out using the SPSS software program (SPSS version 20, IBM, Armonk, NY). The distribution of cases is presented either as absolute values or, in parentheses, as relative frequencies. The Pearson χ^2 test (two-sided) was used to investigate the relationship between SATB2 and other markers, and a *P* value of less than .05 was considered statistically significant. The sensitivity, specificity, and predictive power of various immunohistochemical markers were computed from binary contingency tables, in which the reported diagnosis in the pathologic record was defined as the "true" diagnosis.

Results

IHC-Based Diagnostics

The Department of Surgical Pathology at Uppsala University Hospital receives approximately 30,000 tissue specimens annually for microscopic examination and pathology-based diagnostics. In addition to the assessment of morphologic features evaluated in H&E-stained tissue sections, approximately 10,000 IHC staining procedures are performed each year as part of the diagnostic procedure. Approximately 200 antibodies are used for clinical IHC and thus the pathologist can retrieve additional information to reach a final diagnosis. IHC using CK20 antibodies is mainly performed to confirm or rule out the GI origin of a pathologic process. Between June 2010 and December 2011, CK20 immunostaining was performed in 840 cases. All cases included in this study had SATB2 staining on sections consecutive to the CK20 staining, and the pathologists in charge were requested to ignore the result of SATB2 staining in their diagnostic process.

The mean age of patients included was 67 ± 0.4 years and both sexes were almost equally represented (398 males, 442 females). A mean of seven (range, 4-26) different antibodies was used for each of these 840 cases in which CK20 IHC was deemed necessary in the diagnostic procedure.

Distribution of CK20 and SATB2 in Diagnostic Categories

The final diagnosis, as determined by the pathology reports, was 105 cases of CRC, 458 cases of other C, 179 cases of CUP, and 98 benign cases. The fraction of tumors expressing CK20 and SATB2 were clearly different with respect to the final diagnosis **Figure 1**. A large majority (93%) of CRC cases were CK20 positive, whereas only 13% of other C cases were CK20 positive. In the CUP category, 33% of the tumors were CK20 positive. SATB2 was detected in 93% of CRC cases, 23% in other C cases, and 41% in

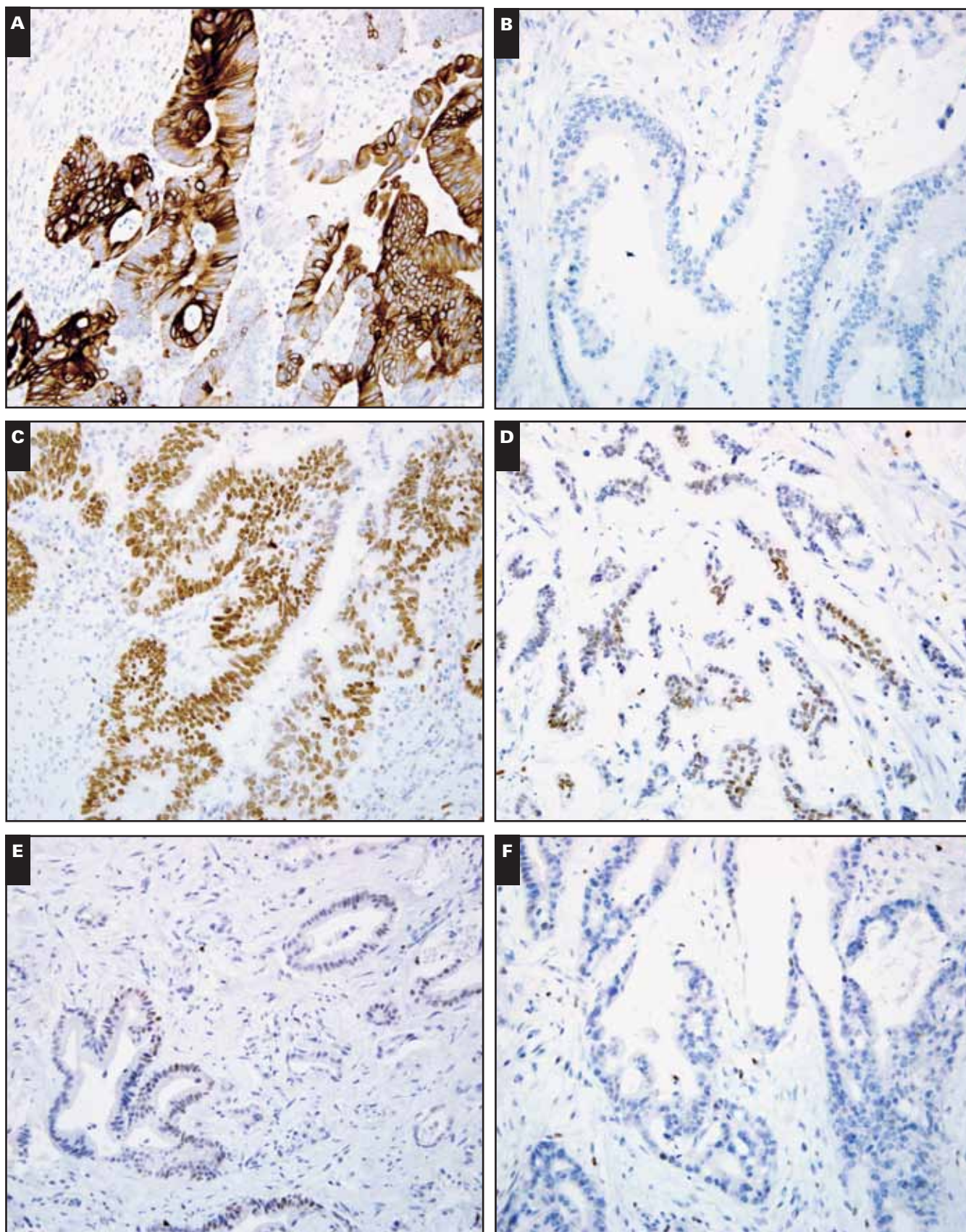


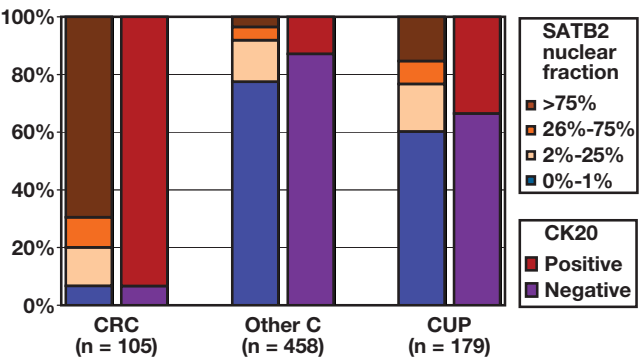
Image 1 Typical examples of CK20 and SATB2 staining in primary colorectal tumors. **A**, CK20 positive. **B**, CK20 negative. **C**, SATB2 diffusely positive (more than 75% nuclei stained, nuclear fraction [NF] = 3). **D**, SATB2 partly positive (26%-75% nuclei stained, NF = 2). **E**, SATB2 sparsely positive (2%-25% nuclei stained, NF = 1). **F**, SATB2 negative (0%-1% nuclei stained, NF = 0). (All images $\times 200$.)

CUP cases. CRC cases showed a substantially larger fraction of cases with widespread SATB2 staining (ie, NF score ≥ 2) compared with other C cases (69% vs 3.5%). Altogether, among the 105 CRC cases, 93 were positive for SATB2, 93 were positive for CK20, and 103 were positive for either marker. A significant correlation was noted between CK20 and SATB2 staining both when all tumors in the study were considered ($P < .01$) and separately for metastases ($P < .01$), the groups of CRC ($P < .05$), “other C” ($P < .01$), and CUP ($P < .01$).

Sensitivity and Specificity for CRC

For computing sensitivity and specificity in binary contingency tables, the SATB2 scores were dichotomized into negative and positive scores. The receiver operator characteristic (ROC) curve for SATB2 as a predictor of CRC was plotted (data not shown) and indicated an optimum for NF between 1 and 2. The cutoff value for SATB2-positive tumors in this study was set at an NF of 1 or more. The sensitivity and specificity were calculated after excluding the CUP and benign cases. The SATB2 marker alone had 93% sensitivity and 77% specificity in determining CRC (Table 1). The CK20 marker alone showed 93% sensitivity and 88% specificity. Similar results were observed when only metastases were analyzed (Table 1).

Because of the reported low specificity of the CK20 marker, in clinical practice the presumed diagnosis of a cancer of colorectal origin is usually confirmed by a combination of two IHC staining responses: expression of CK20 and absence



■Figure 1■ The expression of SATB2 and CK20 in the main categories of tumors expressed as relative percentage. The number of tumors in each category is indicated in parentheses after the tumor type. CRC, colorectal cancer; Other C, certain cancers other than colorectal; and CUP, cancer of unknown primary origin. The results of SATB2 staining are indicated by the fraction of tumor nuclei stained as described in the Materials and Methods section.

of CK7 staining, that is, the immune phenotype CK20+/CK7-. This parallel staining was performed in 636 of the 840 tumor cases, of which 313 were metastases. For our study, the overall sensitivity of the CK20+/CK7- phenotype in diagnosing CRC was 85% and the specificity was 99% (Table 2). For the combination SATB2+/CK7-, the sensitivity was 87% and specificity was 94%. When a triple combination of markers was analyzed,

■Table 1■ Sensitivity and Specificity of a Single Immune Staining in Diagnosis of CRC

| | All Tumors | | | Metastases Only | | |
|----------------|------------|--------|----------------|-----------------|--------|----------------|
| | CK20+ | SATB2+ | CK20+ or SATB2 | CK20+ | SATB2+ | CK20+ or SATB2 |
| Sensitivity, % | 93 | 93 | 98 | 96 | 94 | 99 |
| Specificity, % | 88 | 77 | 71 | 87 | 82 | 73 |
| Positive PP, % | 64 | 49 | 44 | 79 | 72 | 65 |
| Negative PP, % | 98 | 98 | 99 | 98 | 96 | 99 |

CRC, colorectal carcinoma; PP, predictive power.

■Table 2■ Sensitivity and Specificity of a Combined Immune Staining in Diagnosis of CRC

| | All Tumors | | | Metastases Only | | |
|----------------|----------------|-----------------|-------------------------|-----------------|-----------------|-------------------------|
| | CK7- and CK20+ | CK7- and SATB2+ | CK7-, CK20+, and SATB2+ | CK7- and CK20+ | CK7- and SATB2+ | CK7-, CK20+, and SATB2+ |
| Sensitivity, % | 85 | 87 | 83 | 88 | 90 | 87 |
| Specificity, % | 99 | 94 | 100 | 98 | 94 | 99 |
| Positive PP, % | 93 | 76 | 99 | 95 | 88 | 98 |
| Negative PP, % | 97 | 97 | 96 | 95 | 95 | 94 |

CRC, colorectal carcinoma; PP, predictive power.

the phenotype CK7–/CK20+/SATB2+ had a sensitivity of 83% and a specificity of 100% in determining CRC. A similar trend was observed when metastases were analyzed (Table 2).

For 182 cases, immunostaining for CDX2 was performed as part of the diagnostic procedure and the results were retrieved from the pathology reports. The sensitivity of this marker alone in distinguishing CRC cases was 96% and its specificity was 80%. A comparison showing the results of both SATB2 and CDX2 staining is presented in **Table 3**. The cases with the SATB2+/CDX2– phenotype had only scant nuclear expression of SATB2, that is, NF was 1 in both cases of CRC and in four of eight cases of other cancers.

CK20 and SATB2 Staining in Other C Cases

As expected, a fraction of the 458 other C cases showed positive staining for CK20 and/or SATB2 **Table 4**. CK20 was positive in 59 cases (13%) and SATB2 was positive in 102 cases (22%), including all 59 CK20-positive cases. The majority (65 cases) of SATB2-positive other C cases only showed scant nuclear staining. Altogether, only a small fraction (16/458 cases, 3.5%) of other C tumors had widespread expression of SATB2, that is, an NF of 3. These included neuroendocrine tumors (four cases), renal/urothelial cancers (four cases), Merkel cell cancer in skin (three cases), tumors of the small intestine (two cases), and one case each of lung and gynecologic cancer.

CUP Cases

The CUP cases (179 patients) were followed up at intervals of 6 to 24 months after the initial CUP diagnosis and a new diagnosis was available for 113 patients (63%). Also at this time, the pathologist in charge made a final diagnosis unaware of the results of the SATB2 staining. Most common origin for the resolved CUP was the lung (25 cases), followed by pancreatobiliary area (19 cases), neuroendocrine glands (12), and stomach (11 cases), while other organs were represented in very few cases **Table 5**.

Of the cases that were classified finally, 15 were diagnosed as CRC, of which 11 (73%) were CK20+/SATB2+. In two of the remaining CRC cases, the tumor was SATB2+ and CK20–. Among the other resolved CUP cases that were not CRC (98 patients), 22 (23%) expressed CK20 and 29 (30%) expressed SATB2, thus fractions of both SATB2 and CK20 positivity were slightly higher than in the other C group.

Discussion

CRC is the third most common cancer diagnosed globally, with prevalence in the general population reported to be 20 of 100,000 men and 14 of 100,000 women.¹⁴ The diagnosis is based on morphologic examination with the microscope combined with clinical information on the anatomic site of the tumor. In cases with first presentation of a tumor from a

Table 3
Distribution of SATB2 and CDX2 Staining Results in the Colorectal and Noncolorectal Carcinoma (Other C)^a

| | SATB2+ and CDX2+ | SATB2+ and CDX2– | SATB2– and CDX2+ | SATB2– and CDX2– |
|---------------------|------------------|------------------|------------------|------------------|
| Colorectal (n = 51) | 46 | 2 | 3 | 0 |
| Other C (n = 64) | 4 | 8 | 8 | 44 |

^a The number of cases in each organ category is given in parentheses.

Table 4
Distribution of the Diagnoses and Staining Results in the Noncolorectal Carcinoma (Other C)^a

| Cancer Type | SATB2 | | | | CK20 | |
|---------------------------|----------|---------|---------|--------|----------|----------|
| | 0%-1% | 2%-25% | 26%-75% | >75% | Negative | Positive |
| Lung (n = 186) | 153 (82) | 27 (15) | 4 (2) | 1 (1) | 175 (94) | 11 (6) |
| Gynecological (n = 74) | 63 (85) | 6 (8) | 3 (4) | 1 (1) | 68 (92) | 6 (8) |
| Pancreatobiliary (n = 41) | 32 (78) | 5 (12) | 4 (10) | 0 | 30 (73) | 11 (27) |
| Carcinoid (n = 42) | 27 (64) | 9 (21) | 1 (2) | 4 (10) | 40 (95) | 2 (5) |
| Renal/urologic (n = 31) | 19 (61) | 2 (6) | 5 (16) | 4 (13) | 16 (52) | 15 (48) |
| Prostate (n = 22) | 18 (82) | 4 (18) | 0 | 0 | 20 (91) | 2 (9) |
| Gastroesophageal (n = 9) | 7 (78) | 2 (22) | 0 | 0 | 4 (44) | 5 (56) |
| Breast (n = 13) | 11 (85) | 2 (15) | 0 | 0 | 13 (100) | 0 |
| Pleura (n = 6) | 3 (50) | 2 (33) | 1 (17) | 0 | 6 (100) | 0 |
| Small intestine (n = 3) | 0 | 0 | 1 (33) | 2 (67) | 0 | 3 (100) |
| Others (n = 31) | 18 (58) | 6 (19) | 2 (6) | 4 (13) | 27 (87) | 4 (13) |

^a Data are given as number of cases (% of cases relative to the cancer type).

suspected metastatic site or cases with morphology deviating from the expected, IHC can be used to detect the specific protein expression patterns commonly found in both normal colorectal mucosa and CRC.⁵ The most common markers for tumors of colorectal origin include the expression of CK20, often in combination with lack of CK7, that is, the CK20+/CK7– phenotype. However, using this phenotype alone would misdiagnose a proportion of cases, because CK7 is expressed in 10% to 27% of CRCs,^{15,16} and focally in up to 22% of normal colonic mucosa,^{16,17} making it difficult to interpret results in a subset of cases.

In an earlier study, based on the Human Protein Atlas (www.proteinatlas.org),⁸ the expression pattern of the SATB2 protein was analyzed in a multitude of normal human and cancer tissues. In normal epithelial tissues, SATB2 protein was specifically expressed in the nuclei of epithelial cells in the lower GI tract. In nonepithelial cell types, SATB2 was expressed in a subset of lymphoid cells, germ cells in the testis, and certain neurons in the central nervous system. The selective expression of SATB2 in the lower GI tract suggested that SATB2 could function as a diagnostic marker for CRC; therefore this potential diagnostic biomarker was analyzed in a multitude of CRCs and other cancer types. Altogether 1,882 cases of CRC and 620 other, non-CRC tumors were analyzed in one study.⁸ SATB2 was shown to be a sensitive and highly specific marker for CRC, with a distinct positivity seen in 85% of all CRCs. SATB2 is a protein involved in transcription regulation and shows nuclear staining, therefore, it could have some advantages over cytoplasmic/membrane stains such as cytokeratins. Staining of a nuclear transcription factor should in theory result in an “all or none” pattern and should be less sensitive to the degree of tumor differentiation.¹⁷ In this prospective study, we investigated the diagnostic value of SATB2 in a true clinical setting. For purposes of simplicity in the annotation/diagnostic process and to facilitate comparison with similar studies, the

scoring for SATB2 NF in this study was set for intuitive intervals of 0% to 1% (NF = 0), 2% to 25% (NF = 1), 26% to 75% (NF = 2), and more than 75% (NF = 3) of tumor cells.

In our prospective cohort, CK20 was ubiquitously expressed in the cases diagnosed with CRC and showed a calculated sensitivity of 93%. The specificity of CK20 alone in diagnosing CRC was 88% in our study. This differs slightly from other published data on CK20 expression in CRCs, which report sensitivity ranging from 65% to 86%.¹⁸⁻²⁰ The difference could be related to the variability in the definition of positive marker, that is, in some studies (as in ours), any CK20 staining was considered positive, whereas in others, a minimum of 5% or 10% stained tumor cells was required for positive classification. The difference could also be related to an inherent bias selection of cases included in different studies. The current study reflects the actual clinical situation, so patients in this study are not representative of all cancer cases arising in the normal population nor of all cancer cases diagnosed at a pathology clinic in a population-based regional hospital; this is because the main inclusion criterion was the need to perform CK20 immunohistochemical staining to obtain a diagnosis. This decision was taken individually by the pathologist diagnosing each incoming case. Although the differentiation grade of the tumor or the difficulties in making a diagnosis were not recorded in the current study, we can assume that the 840 cases included in this study were not the most common, standard forms of tumors. This is because the cohort includes only a small fraction (8%) of all cancers diagnosed at our clinic during the study period (which is approximately 10,000 cancer cases per year). Further, IHC using an average of seven different antibodies was required for each case to make a diagnosis. Moreover, our prospective cohort included CUPs in a substantially larger proportion (21%) than the 3% expected in the average population.¹ Despite not representing the general patient population, the added value of our

Table 5
Distribution of the Diagnoses and Staining Results after Follow-up of Cancer of Unknown Primary (CUP) Cases^a

| Cancer Type | SATB2 | | | | CK20 | |
|---------------------------|---------|--------|---------|--------|----------|----------|
| | 0%-1% | 2%-25% | 26%-75% | >75% | Negative | Positive |
| Still CUP (n = 66) | 39 (59) | 8 (12) | 8 (12) | 9 (14) | 40 (61) | 26 (39) |
| Colorectal (n = 15) | 2 (13) | 3 (20) | 3 (20) | 7 (47) | 3 (20) | 12 (80) |
| Lung (n = 25) | 19 (76) | 5 (20) | 1 (4) | 0 | 23 (92) | 2 (8) |
| Pancreatobiliary (n = 19) | 14 (74) | 4 (21) | 0 | 1 (5) | 15 (79) | 4 (21) |
| Carcinoid (n = 12) | 5 (42) | 3 (25) | 0 | 3 (25) | 8 (67) | 4 (33) |
| Gastroesophageal (n = 11) | 6 (55) | 1 (9) | 1 (9) | 3 (27) | 6 (55) | 5 (45) |
| Gynecological (n = 9) | 7 (78) | 2 (22) | 0 | 0 | 9 (100) | 0 |
| Renal/urologic (n = 6) | 2 (33) | 1 (17) | 1 (17) | 1 (17) | 2 (33) | 4 (67) |
| Skin (n = 4) | 3 (75) | 0 | 0 | 1 (25) | 3 (75) | 1 (25) |
| Small intestine (n = 3) | 1 (33) | 1 (33) | 0 | 1 (33) | 2 (67) | 1 (33) |
| Breast (n = 3) | 3 (100) | 0 | 0 | 0 | 3 (100) | 0 |
| Others (n = 4) | 3 (75) | 1 (25) | 0 | 0 | 4 (100) | 0 |

^a Data are given as number of cases (% of cases relative to the cancer type).

study is that it is truly representative of the diagnostic work at a clinical department in a university hospital.

In our study, SATB2 was widely expressed in the colorectal cases, with 93% of cases staining at least 2% of tumor cells. The correlation between CK20 and SATB2 was statistically significant when all cases were considered and also separately for metastases, CRC cases, other C cases, and CUPs. In the study by Magnusson et al,⁸ which included more than 1,800 patients with CRC in total, SATB2 was positive in 85% of CRCs. Unlike our prospective cohort, the 1,800 patients with CRC and the other tumor types in that study were selected on a retrospective basis and included conventional tumor cases. In a recent study that prospectively included incident CRC cases without further selection 72% of CRC cases were SATB2+.¹⁰ The specificity of SATB2 for determining a CRC diagnosis was lower in our prospective study compared with the retrospective study of Magnusson et al,⁸ which included 620 typical cases of cancers of various organs and showed the specificity of SATB2 to be high: no prostate cancers were positive and less than 5% of lung, breast, ovarian, gastric, and pancreatobiliary adenocarcinoma were positive. In our study, 15% of conclusive cancers (“other C”) expressed CK20 and 23% had detectable SATB2 (NF = 1), though most cases showed only sparse staining (NF = 1). Only a small fraction of these non-CRC tumors (3.5%) showed diffuse expression of SATB2 (ie, NF = 3). Except for lung cancer, the number of cases in each type of cancer was small, and thus the selection of cancer types in our cohort cannot be considered representative of other specific tumor types.

In 10 (9.4%) of 105 colorectal cases, the results of CK20 and SATB2 staining were incongruent, with five cases being CK20+/SATB2– and five cases CK20–/SATB2+. Of importance in these cases was the fact that the primary tumor was a poorly differentiated adenocarcinoma (5 cases), or in the cases with medium differentiation, the tumor was a rectal cancer (3 cases). It is important to note that in the cases with the CK20–/SATB2+ phenotype, the pathologist reached the diagnosis of CRC by other means (usually other immune stains in combination with the clinical context) without considering the result of SATB2 staining; this indicates that SATB2 staining could yield an added value in the diagnostic algorithm.

Discrepancies were seen between CK20 and SATB2 even among the other C diagnoses: 29 cases had the CK20+/SATB2– phenotype and 76 cases had the opposite phenotype, CK20–/SATB2+ (although only 7 cases had only a diffuse SATB2 staining). Although we can speculate on the accuracy of the diagnosis in these cases, because of the constraints of our study, a review of the diagnostic material was not possible. The diagnosis in these discordant cases was retrieved again from the pathology clinical database at follow-up and no relevant change was noted. This reassures us that the adherence to the current national diagnostic guidelines together with the pathologists’

access to radiologic/clinical information and ancillary analyses were sufficient for a “state-of-the art” diagnosis.

Any diagnostic marker’s value includes its sensitivity and specificity in determining the diagnosis in question. In the literature, the sensitivity of the classic phenotype CK7–/CK20+ in diagnosing CRC is reported between 64% and 78% and its specificity between 65% and 98%.^{5,18,21} Because positive CK20 staining, in combination with negative CK7 staining, is commonly used to determine a diagnosis of CRC in difficult cases, this phenotype will be inherently overrepresented in the CRC category. In our study, the CK7–/CK20+ phenotype showed a remarkable specificity of 99%, whereas the CK7–/SATB2+ phenotype showed specificity close to that of the classic CRC phenotype (94%). It might be argued that some cases were diagnosed as CRC based solely on the CK7–/CK20+ immunophenotype; however, in most cases, the pathologist in charge had information from the radiologic and surgical reports, as well as ancillary analyses. Moreover, the recheck at follow-up did not indicate any relevant change in the initial diagnosis of these patients.

The sensitivity of the combination of markers CK7–/SATB2+ (87%) was marginally better than that of the classic combination of CK7–/CK20+ (85% in our study), and the specificity was slightly inferior, both for primary tumors and for metastases (Table 2). In an analysis of a triple combination of markers, the CK7–/CK20+/SATB2+ phenotype was found to have a sensitivity of 83% and a specificity of 100% in determining the colorectal origin.

Both the distribution of the staining scores in various cancer types and the ROC for predicting CRC based on SATB2 NF point to a need for setting the diagnostic threshold for positive SATB2 tumors at a value between 2% and 25% stained tumor cells. When the threshold for SATB2+ tumors was set at an NF of 2 or more, the specificity of SATB2 alone (92%) was superior to that of the classic marker CK20 alone (88%), but with a small decrease in sensitivity (data not shown). The optimal threshold value remains to be further determined.

The pattern of expression for CK20 and CK7 was previously reported to be associated with the cancer type: diffuse CK20 staining in CRC vs focal CK20 staining in gastric/pancreatic carcinoma and the opposite for CK7.²¹ For SATB2, the pattern of expression can be inferred from the NF, with any NF less than 2 indicating a patchy staining. This score was observed more often in other C cases than in CRCs, a fact that could help in guiding the clinical diagnosis.

In addition to the classic cytokeratins, antibodies against CDX2 (caudal-type homeobox 2) are often used as markers of GI origin. In normal tissues, CDX2 expression is restricted to glandular cells from the proximal duodenum to distal rectum. The sensitivity of CDX2 for CRC is high, but there is a lack of specificity because a substantial fraction of pancreatobiliary

carcinoma and carcinoma of the stomach, small intestine, esophagus, and ovary can be positive for CDX2.²¹⁻²⁴

Only 182 of 840 cases were stained for CDX2, which reflected the fact that in the current clinical study, CDX2 IHC was not deemed necessary in a majority of the cases for making a final diagnosis. The calculated sensitivity and specificity for CRC diagnosis using CDX2 were 96% and 80%, respectively, which were similar to those of CK20 and SATB2. In the study of Magnusson et al,⁸ which analyzed 245 standard cases of primary and metastatic CRC, 95% of cases were positive for CDX2 and the specificity is not given. High specificity of CDX2—of up to 100% for diagnosing metastases of CRC to the lung, as reported by Barbareschi et al²⁵—may be because of case selection (that study did not include any cases of metastases from other relevant GI carcinomas). In contrast, our study included the cases prospectively, and the observed sensitivity and specificity are more likely to reflect authentic diagnostic practices in the clinic.

In conclusion, we present herein the first unbiased data on the application of SATB2 as a diagnostic marker for CRC in a clinical prospective cohort. Our results confirm the high sensitivity and specificity of SATB2 in a clinical context, and suggest that immunohistochemical staining with antibodies to SATB2 can add important information to confirm or rule out tumors of colorectal origin.

Address reprint requests to Dr Dragomir: Dept of Immunology, Genetics and Pathology, Uppsala University, 75185 Uppsala, Sweden; anca.dragomir@igp.uu.se.

This work was supported by grants from the Knut and Alice Wallenberg Foundation and from Uppsala University.

Acknowledgments: The authors acknowledge the pathologists and entire staff of the Department of Surgical Pathology, Uppsala University Hospital, Uppsala, Sweden, for providing the excellent clinical diagnostic work that forms the basis of this study.

References

- Pavlidis N, Briassoulis E, Hainsworth J, et al. Diagnostic and therapeutic management of cancer of an unknown primary. *Eur J Cancer*. 2003;39:1990-2005.
- Finishing the euchromatic sequence of the human genome. *Nature*. 2004;431:931-945.
- Pontén F, Gry M, Fagerberg L, et al. A global view of protein expression in human cells, tissues, and organs. *Mol Syst Biol*. 2009;5:337.
- Moll R, Schiller DL, Franke WW. Identification of protein IT of the intestinal cytoskeleton as a novel type I cytokeratin with unusual properties and expression patterns. *J Cell Biol*. 1990;111:567-580.
- Tot T. Cytokeratins 20 and 7 as biomarkers: usefulness in discriminating primary from metastatic adenocarcinoma. *Eur J Cancer*. 2002;38:758-763.
- Dennis JL, Hvidsten TR, Wit EC, et al. Markers of adenocarcinoma characteristic of the site of origin: development of a diagnostic algorithm. *Clin Cancer Res*. 2005;11:3766-3772.
- Su MC, Yuan RH, Lin CY, et al. Cadherin-17 is a useful diagnostic marker for adenocarcinomas of the digestive system. *Mod Pathol*. 2008;21:1379-1386.
- Magnusson K, de Wit M, Brennan DJ, et al. SATB2 in combination with cytokeratin 20 identifies over 95% of all colorectal carcinomas. *Am J Surg Pathol*. 2011;35:937-948.
- Wang S, Zhou J, Wang XY, et al. Down-regulated expression of SATB2 is associated with metastasis and poor prognosis in colorectal cancer. *J Pathol*. 2009;219:114-122.
- Eberhard J, Gaber A, Wangefjord S, et al. A cohort study of the prognostic and treatment predictive value of SATB2 expression in colorectal cancer. *Br J Cancer*. 2012;106:931-938.
- Chung J, Lau J, Cheng LS, et al. SATB2 augments DeltaNp63alpha in head and neck squamous cell carcinoma. *EMBO Rep*. 2010;11:777-783.
- McGovern DP, Gardet A, Torkvist L, et al. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. *Nat Genet*. 2010;42:332-337.
- Kampf C, Olsson I, Ryberg U, et al. Production of tissue microarrays, immunohistochemistry staining and digitalization within the human protein atlas. *J Vis Exp*. 2012;63(pii): 3620.
- Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;55:74-108.
- Hernandez BY, Frierson HF, Moskaluk CA, et al. CK20 and CK7 protein expression in colorectal cancer: demonstration of the utility of a population-based tissue microarray. *Hum Pathol*. 2005;36:275-281.
- Zhang PJ, Shah M, Spiegel GW, et al. Cytokeratin 7 immunoreactivity in rectal adenocarcinomas. *Appl Immunohistochem Mol Morphol*. 2003;11:306-310.
- Saad RS, Silverman JF, Khalifa MA, et al. CDX2, cytokeratins 7 and 20 immunoreactivity in rectal adenocarcinoma. *Appl Immunohistochem Mol Morphol*. 2009;17:196-201.
- Bayrak R, Yenidunya S, Haltas H. Cytokeratin 7 and cytokeratin 20 expression in colorectal adenocarcinomas. *Pathol Res Pract*. 2011;207:156-160.
- Torenbeek R, Lagendijk JH, Van Diest PJ, et al. Value of a panel of antibodies to identify the primary origin of adenocarcinomas presenting as bladder carcinoma. *Histopathology*. 1998;32:20-27.
- Tot T. Adenocarcinomas metastatic to the liver: the value of cytokeratins 20 and 7 in the search for unknown primary tumors. *Cancer*. 1999;85:171-177.
- Bayrak R, Haltas H, Yenidunya S. The value of CDX2 and cytokeratins 7 and 20 expression in differentiating colorectal adenocarcinomas from extraintestinal gastrointestinal adenocarcinomas: cytokeratin 7-/20+ phenotype is more specific than CDX2 antibody. *Diagn Pathol*. 2012;7:9.
- Saad RS, Ghorab Z, Khalifa MA, et al. CDX2 as a marker for intestinal differentiation: its utility and limitations. *World J Gastrointest Surg*. 2011;3:159-166.
- Tot T. Identifying colorectal metastases in liver biopsies: the novel CDX2 antibody is less specific than the cytokeratin 20+/7- phenotype. *Med Sci Monit*. 2004;10:BR139-143.
- Werling RW, Yaziji H, Bacchi CE, et al. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol*. 2003;27:303-310.
- Barbareschi M, Murer B, Collby TV, et al. CDX-2 homeobox gene expression is a reliable marker of colorectal adenocarcinoma metastases to the lungs. *Am J Surg Pathol*. 2003;27:141-149.

First and Only FDA Cleared Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager



Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer
with the Advanced Technology of the
Genius™ Digital Diagnostics System



Click or Scan
to discover more

ADS-04159-001 Rev 001 © 2024 Hologic, Inc. All rights reserved. Hologic, Genius, and associated logos are trademarks and/or registered trademarks of Hologic, Inc. and/or its subsidiaries in the United States and/or other countries. This information is intended for medical professionals in the U.S. and other markets and is not intended as a product solicitation or promotion where such activities are prohibited. Because Hologic materials are distributed through websites, podcasts and tradeshows, it is not always possible to control where such materials appear. For specific information on what products are available for sale in a particular country, please contact your Hologic representative or write to diagnostic.solutions@hologic.com.

genius™
DIGITAL DIAGNOSTICS