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Cadherin-17 and SATB2 Are Sensitive and Specific Immunomarkers for Medullary Carcinoma of the Large Intestine

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• ***Context.***—**Distinction of medullary carcinoma of the large intestine from other cytokeratin (CK) 7$^-$/CK20$^-$ carcinomas can be challenging when working on a tumor of unknown primary because the majority of medullary carcinomas are negative for CK7, CK20, and CDX2.**

Objective.—**To investigate the expression of cadherin-17 and SATB-2 and other markers in medullary carcinomas of the large intestine and cadherin-17 and SATB2 in a large number of carcinomas and normal tissues from various organs to further test their diagnostic specificity.**

Design.—**This study evaluated cadherin-17 and SATB2 expression in 18 medullary carcinoma cases and 1941 tumors and 358 normal tissues from various organs. Other immunomarkers, including MLH1, PMS2, MSH2, MSH6, CDX2, CK7, CK20, TFF3, MUC4, calretinin, p504S, villin, and synaptophysin, were also tested on the 18 medullary carcinoma cases.**

Results.—**The results demonstrated (1) loss of MLH1 and PMS2 in more than 80% of medullary carcinomas; (2) expression of cadherin-17 and SATB2 in 89% of medullary carcinomas; (3) focal expression of TFF3, MUC4, calretinin, CDX2, CK20, and synaptophysin in 72%, 72%, 67%, 67%, 28%, and 17% of 18 medullary carcinoma cases, respectively; and (4) expression of SATB2 and cadherin-17 in 97% and 98% of the colorectal adenocarcinomas, respectively, whereas their expression was seen in 3.6% and 3.3% of nongastrointestinal tumors, respectively.**

Conclusions.—**We concluded that SATB2 and cadherin-17 were highly sensitive and specific markers for colorectal carcinomas and propose including MLH1, cadherin-17, and SATB2 in a routine immunostaining panel when working on a tumor of unknown primary, especially in an elderly patient with a CK7$^-$/CK20$^-$ carcinoma.**

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Medullary carcinoma (MC) of the colon and rectum is a rare, distinct variant of colorectal carcinoma first described nearly 2 decades ago.[1–6] The World Health Organization recognizes MC as a separate entity of colorectal carcinoma in the 2010 edition of *WHO Classification of Tumours of the Digestive System*.[7] Medullary carcinoma typically presents as a large-sized tumor of the right side of the colon, especially in the cecum, in elderly patients, and is more frequently seen in women than in men, with a ratio of 2:1. Histologically, MC presents with the following features: solid sheets, nests, or trabeculae of intermediate to large polygonal tumor cells with high nuclear/cytoplasmic ratio; vesicular nuclear chromatin and conspicuous to prominent nucleoli; amphophilic cytoplasm; pushing tumor border; peri– and intra–tumor cell lymphocytic infiltration; and minimal or no glandular formation. Medullary carcinoma tends to present with a high tumor stage (T3 or T4) but has a favorable prognosis (less lymph node and distant metastasis) compared with poorly differentiated adenocarcinoma or neuroendocrine carcinoma of the large intestine.[1–9]

In molecular aspects, microsatellite instability (MSI) has been reported in approximately 80% of MC cases, with the majority of cases negative for both MLH1 and PMS2.[1,3,5,8,9] The evidence of MSI in MC is usually associated with a sporadic event rather than a hereditary nonpolyposis colorectal cancer and is secondary to a promoter hypermethylation.[5] Other molecular findings in MC include lack of p53 overexpression, diploid DNA, and no evidence of adenomatous polyposis coli, deleted in colorectal cancer or β-catenin mutations.[1,5,9] In contrast, the mutations of these genes are identified in 80% of colorectal cancers, indicating a different molecular pathway of tumorigenesis involving MC.[1,5,9]

In regards to immunohistochemistry, only a limited number of reports are available. It has been well established

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Dr Li and Dr T. Chen are employees of Epitomics, Inc, an Abcam Company, in Burlingame, California; antibodies CDH17 (clone EP86), SATB2 (clone EP281 or EPNCIR130B), and TFF3 (EP107) are products of Epitomics. The other authors have no relevant financial interest in the products or companies described in this article.

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that caudal type homeobox 2 (CDX2) is a highly sensitive immunomarker for gastrointestinal (GI) adenocarcinomas but may also be expressed in tumors from other organs, such as the pancreas, bile ducts, bladder, uterine cervix, endometrium, and ovary.[10–15] Marked reduction or loss of expression of cytokeratin 20 (CK20) and CDX2 is frequently seen in MC.[9,16–19] Wick and colleagues[20] reported that multifocal positivity of neuroendocrine markers (synaptophysin and chromogranin A) and focal p53 positivity were observed in 32% and 53% of MC cases, respectively. Winn et al[16] reported that a high percentage of MC cases were positive for mucin 1 (MUC1), mucin 2 (MUC2), and trefoil factor 3 (TFF3), indicating intestinal differentiation. Additionally, Winn et al[16] reported that 73% of MC cases were positive for calretinin, which can potentially be used as a diagnostic marker for MC.[16] To complicate this matter further, our experience and others' have demonstrated that a small percentage of MCs can be positive for cytokeratin 7 (CK7).[16,21] As one can imagine, when an MC presents as a tumor of unknown primary, the pathologist may misinterpret it, based on its unusual histologic and immunophenotypic features, as another $CK7^+$ carcinoma, or as a mesothelioma because of CK7 and calretinin positivity; or the pathologist may simply exclude a colorectal primary because of the absence of CK20 and CDX2 expression. Therefore, there is a need to search for more sensitive and specific markers to confirm a diagnosis of MC of the large intestine.

Cadherin-17 (CDH17), also known as Li-cadherin (liver-intestine cadherin), is a member of the cadherin superfamily and is a calcium-dependent transmembrane glycoprotein.[22–25] The main function of CDH17 is to mediate cell-cell adhesion and to act as an intestinal peptide transporter.[22–25] CDH17 is expressed in normal glandular epithelium of the GI tract and normal pancreatic ducts.[25] A limited number of reports have demonstrated that CDH17 is a highly sensitive marker for GI adenocarcinomas and neuroendocrine neoplasms.[24,25] Panarelli et al[25] have also reported that CDH17 was a more sensitive marker than CDX2 in identification of GI adenocarcinomas and was observed in only a small percentage of adenocarcinomas of the lung, breast, ovary and endometrium. However, CDH17 expression in MC of the large intestine has not been reported in the literature.

Special AT-rich sequence binding protein 2 (SATB2) is another recently described marker that functions as a nuclear matrix-associated transcription factor and an epigenetic regulator.[26,27] Magnusson and coworkers[27] have reported that SATB2 in combination with CK20 can identify more than 95% of all colorectal carcinomas. Upper GI carcinomas and pancreatic adenocarcinomas are usually negative for SATB2, and ovarian carcinomas and lung adenocarcinomas are positive for SATB2 with low frequency.[27] Therefore, SATB2 is a potential marker for identifying a carcinoma of colorectal origin when working on a tumor of unknown primary.[27] SATB2 expression in MC of the large intestine has not been reported in the literature.

In the present study, we investigated (1) the expression of CDH17 and SATB2 in MC of the large intestine; (2) the expression of other markers of intestinal differentiation such as CDX2, CK20, TFF3, villin, mucin 4 (MUC4), and α-methylacyl-CoA racemase (AMACR/P504S) in MC; (3) the expression of previously reported markers such as MSI markers, calretinin, and synaptophysin in MC; and (4) the expression of CDH17 and SATB2 in a large number of carcinomas and normal tissues from various organs, including some organs that have not been extensively studied in the literature, such as kidney, bladder, liver, endocervix, endometrium, thyroid, and ovary, to further test the diagnostic specificity of these 2 markers.

MATERIALS AND METHODS

Identification of MC Cases

The study was conducted with approval from the Institutional Review Board of Geisinger Health System. The pathologists at Geisinger began to use the terminology *medullary carcinoma of the colon and rectum* in early 2000. We searched the Geisinger surgical pathology files from the years 2000 through 2013 for resection specimens of colorectal carcinomas using the key words "adenocarcinoma/carcinoma, colon, resection," "medullary carcinoma," "poorly differentiated carcinoma with medullary features," and "undifferentiated carcinoma with medullary features," and 1114 cases of colorectal carcinoma were identified. Among those cases, 18 (1.6%) were diagnosed as MCs or poorly differentiated/undifferentiated carcinomas with medullary features. The hematoxylin-eosin–stained slides of the 18 MCs were rereviewed by 2 surgical pathologists. The clinical information for these 18 cases is summarized in Table 1.

Construction of Tissue Microarray Blocks

One thousand nine hundred forty-one cases of tumors and 358 cases of normal tissues from various organs dating from 2000 to 2013 were retrieved from the archives of the Department of Laboratory Medicine at Geisinger Medical Center. Multiple tissue microarray blocks with 2 punches of 0.75 or 1.0 mm each for each case were constructed as previously described.[28]

Immunohistochemistry

Immunohistochemical stains were performed on 18 MC cases on routine tissue sections and on 2299 tumors and normal tissues from various organs on tissue microarray slides using the previously published protocol.[28,29] For the 18 MC cases, staining for MSI markers (MLH1, PMS2, mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), CDH17, SATB2, CDX2, CK7, CK20, TFF3, MUC4, calretinin, P504S, villin, and synaptophysin were performed. CDH17 and SATB2 stains were also performed on the tissue microarray slides containing various tumors ($n = 1941$) and normal tissues ($n = 358$). Detailed information about the antibodies and staining conditions is summarized in Table 2. Appropriate positive control tissues were used for each of the above antibodies.

The staining intensity for both tumor cases and normal tissues was graded as weak, intermediate, or strong. In general, a strong signal can be easily seen with low-power magnification (×2 or ×4), an intermediate signal can be observed with ×10 magnification, and a weak signal can be seen with high-power magnification (×20 or ×40). The distribution was recorded as negative (<5% of tumor cells stained), 1+ (5%–25%), 2+ (26%–50%), 3+ (51%–75%), or 4+ (>75%). Two surgical pathologists independently evaluated the immunostained slides. If a disagreement occurred, the 2 pathologists reviewed the case together and reached the final agreement. The interpretation was generally straightforward in this study. The results from the 2 pathologists were very compatible, with no significant disagreements.

RESULTS

Clinical and Histologic Features of 18 MCs

All 18 MC cases demonstrated classic histologic features, including solid sheets, nests, or trabeculae of intermediate to large polygonal tumor cells with high nuclear to cytoplasmic ratio; vesicular nuclear chromatin and conspicuous to prominent nucleoli; amphophilic cytoplasm; pushing or focal infiltrating tumor border; dense peri– and intra–tumor

Table 1. Summary of Clinical Information and Microsatellite Instability (MSI) Status for 18 Medullary Carcinomas[a]

Case	Sex	Age	Location	Tumor Size (cm)	TNM	MSI Status
1	M	94	Cecum	10.0	T3N1Mx	No evidence of MSI
2	F	80	Cecum	6.4	T3N1bMx	Loss of MLH1 and PMS2
3	F	80	Cecum	10.5	T4aN1aMx	Loss of MLH1 and PMS2
4	F	80	Ascending	7.5	T3N0Mx	Loss of MLH1 and PMS2
5	F	67	Cecum	6.5	T3N0Mx	Loss of MSH2 and MSH6
6	F	89	Ascending	9.5	T3N1Mx	No evidence of MSI
7	M	72	Transverse	10.5	T4bN0Mx	No evidence of MSI
8	F	72	Cecum	7.5	T3N0Mx	Loss of MLH1 and PMS2
9	M	88	Cecum	4.0	T3N0Mx	Loss of MLH1 and PMS2
10	F	80	Cecum	9.0	T3N0Mx	Loss of MLH1 and PMS2
11	F	72	Ascending	10.5	T3NoMx	Loss of MLH1 and PMS2
12	F	86	Cecum	7.0	T3N0Mx	Loss of MLH1 and PMS2
13	M	84	Ascending	10.1	T3N0Mx	Loss of MLH1 and PMS2
14	F	82	Cecum	15.0	T4bN1bM1	Loss of MLH1 and PMS2
15	F	91	Cecum	5.8	T3N0Mx	Loss of MLH1 and PMS2
16	F	52	Ascending	10.5	T3N0Mx	Loss of MLH1 and PMS2
17	M	78	Cecum	10.0	T3N1Mx	Loss of MLH1 and PMS2
18	F	87	Cecum	3.5	T3N0Mx	Loss of MLH1 and PMS2

[a] Case 7 also presented with a 6.0 cm well-differentiated adenocarcinoma in the sigmoid colon with no evidence of MSI; case 16 was positive for lymphovascular invasion; case 14 presented with a liver metastasis by computed tomography scan; case 2 also presented with a tubulovillous adenoma; case 17 also presented with multiple tubular adenomas; and case 13 presented with a 10.0-cm left-side colon/mesenteric metastasis 6 months after initial right-side colectomy.

cell lymphocytic infiltration; and minimal or no glandular formation. The ratio of women to men was 2.6:1. The mean age was 79.7, with a range from 52 to 91. The carcinomas were located in the cecum (n = 12; 67%), ascending colon (n = 5; 27%), and transverse colon (n = 1; 6%). The average tumor size was 8.5 cm, with a range from 3.5 to 15 cm. There were 8 cases of metastatic disease, including 6 cases in the regional lymph nodes, 1 case with liver metastasis (case 14), and 1 case with left-side colon/mesenteric metastasis (case 13). Clinical follow-up data could not be obtained in these cases. The detailed information for each case is summarized in Table 1.

Immunohistochemical Staining Results on 18 MCs

Fifteen of 18 MC cases (83%) demonstrated loss of expression of the MSI markers. Among these 15 cases, 14 showed loss of both MLH1 and PMS2, and 1 showed loss of MSH2 and MSH6. In all of the MSI cases, the adjacent normal colonic mucosa and internal control tissue (lymphoid cells and stromal cells) were strongly positive for all 4

Table 2. Summary of Antibody Information and Staining Conditions

Antibody	Vendor[a]	Catalog No.	Approved Use	Clonality	Host Animal	Dilution	Incubation Time, min	Antigen Retrieval[b]			Localization
								Method	Time, min	Temperature, °C	
MLH1	Ventana	790-4535	IVD	M1	Mouse	Predilute	24	CC1	64	95	N
PMS2	Cell Marque	288R-18	IVD	EPR3947	Rabbit	Predilute	32	CC1	64	95	N
MSH2	Ventana	760-4265	IVD	G219-1129	Mouse	Predilute	32	CC1	36	95	N
MSH6	Cell Marque	278M-18	IVD	44	Mouse	Predilute	8	CC1	36	95	N
CDH17	Epitomics/ Abcam	AC-0095RUO	RUO	EP86	Rabbit	1:100	32	CC1	36	95	M
SATB2[c]	Santa Cruz	Sc-81376	RUO	SATBA4B10	Mouse	1:20	44	CC1	64	95	N
SATB2[c]	Epitomics/ Abcam	2891-1	RUO	EP281/ EPNCIR130B	Rabbit	1:100	40	EDTA	15	100	N
CDX2	Cell Marque	235R-16	IVD	EPR2764Y	Rabbit	1:600	24	CC1	36	95	N
CK7	Cell Marque	307M-95	IVD	OV-TL 12/30	Mouse	1:200	20	CC1	36	95	C
CK20	Ventana	790-4431	IVD	SP33	Rabbit	Predilute	20	CC1	64	95	C
MUC4	Biocare	CM326A	IVD	8G-7	Mouse	1:200	40	CC1	36	95	C
Calretinin	Ventana	790-4467	IVD	SP65	Rabbit	Predilute	24	CC1	36	95	N + C
P504S	Biocare	PP365JJ	ASR	Polyclonal	Mouse	Predilute	32	CC1	64	95	C
Villin	Ventana	760-4277	IVD	CWWB1	Mouse	Predilute	32	CC1	36	95	M
Synaptophysin	Cell Marque	336R-98	IVD	MRQ-40	Rabbit	Predilute	16	CC1	36	95	C
TFF3	Epitomics/ Abcam	AC-0103	RUO	EP107	Rabbit	1:800	40	CC1	36	95	C

Abbreviations: ASR, analyte-specific reagent; C, cytoplasmic staining; IVD, in vitro diagnostic; M, membranous staining; N, nuclear staining; RUO, research use only.

[a] Biocare Medical, Inc, Concord, California; Cell Marque Corporation, Rocklin, California; Epitomics, an Abcam Company, Burlingame, California; Santa Cruz Biotechnology, Inc, Santa Cruz, California; Ventana Medical Systems, Tucson, Arizona.
[b] pH = 8 for all antigen retrieval procedures.
[c] Similar staining results were obtained by using 2 different antibodies against SATB2.

Table 3. Expression of Cadherin-17 (CDH17), SATB-2, CDX2, Cytokeratin 7 (CK7), Cytokeratin 20 (CK20), MUC4, TFF3, Calretinin, P504S, Synaptophysin, and Villin in 18 Medullary Carcinomas

Case No.	CDH17	SABT-2	CDX2	CK20	Calretinin	TFF3	MUC4	P504S	Villin	CK7	Synapto-physin
1	4+, S	3+, I	4+, I	2+, S	2+, S	2+, S	1+, S	3+, W	Neg	Neg	Neg
2	4+, S	3+, I	4+, I	Neg	Neg	2+, I	Neg	4+, I	Neg	Neg	1+, I
3	4+, S	3+, W	1+, I	Neg	1+, W	1+, S	1+, S	Neg	Neg	Neg	Neg
4	3+, S	Neg	1+, W	Neg	1+, W	4+, S	Neg	Neg	Neg	Neg	Neg
5	4+, S	2+, S	4+, S	Neg	Neg	Neg	2+, S	3+, S	Neg	Neg	1+, W
6	4+, S	4+, S	4+, I	Neg	1+, S	Neg	3+, S	2+, I	Neg	Neg	Neg
7	3+, S	4+, S	1+, I	1+, S	Neg	2+, S	2+, S	2+, W	Neg	Neg	Neg
8	4+, S	3+, S	3+, S	2+, S	2+, S	2+, S	2+, S	3+, I	Neg	Neg	Neg
9	4+, S	4+, I	1+, I	Neg	3+, S	2+, S	1+, S	2+, W	2+, I	Neg	Neg
10	4+, S	4+, I	2+, W	2+, S	Neg	Neg	Neg	2+, I	Neg	Neg	Neg
11	1+, S	2+, I	1+, I	Neg	2+, S	2+, S	Neg	Neg	Neg	Neg	Neg
12	1+, S	4+, I	Neg	Neg	3+, S	1+, S	1+, S	Neg	3+, S	Neg	1+, I
13	Neg	4+, I	Neg	Neg	1+, W	Neg	1+, S	Neg	Neg	3+, S	Neg
14	Neg	4+, S	Neg	Neg	Neg	Neg	Neg	2+, W	Neg	Neg	Neg
15	2+, I	2+, S	1+, W	Neg	3+, S	2+, S	1+, S	Neg	Neg	Neg	Neg
16	3+, S	3+, S	Neg	Neg	1+, S	2+, S	4+, S	Neg	Neg	Neg	Neg
17	4+, S	2+, I	Neg	1+, I	Neg	3+, S	1+, S	2+, I	Neg	Neg	Neg
18	1+, S	Neg	Neg	Neg	1+, S	1+, S	2+, S	Neg	Neg	Neg	Neg
No. positive/ total No. cases (%)	16/18 (89)	16/18 (89)	12/18 (67)	5/18 (28)	12/18 (67); >3+ stain	13/18 (72)	13/18 (72)	10/18 (56)	2/18 (11)	1/18 (6)	3/18 (17)
No. >3+ stain/total No. positive (%)	12/16 (75)	12/16 (75)	5/12 (42)	0	3/12 (25)	2/13 (15)	2/13 (15)	4/10 (40)	1/2 (50)	1/1 (100)	0

Abbreviations: I, intermediate; Neg, negative; S, strong; W, weak.

MSI markers. The immunohistochemical staining results for MSI status of the 18 MC cases are summarized in Table 1.

Only 5 of 18 MC cases (25%) were focally positive (1+ or 2+) for CK20, and 12 of 18 cases were positive for CDX2 (67%). However, the CDX2 positivity tends to be focal (1+ or 2+) or weak staining. Strong CDX2 staining was only seen in 2 of 12 positively stained cases. In contrast, CDH17 was positive in 16 of 18 cases (89%) with diffuse (3+ or 4+) positivity in 75% of the cases. Similarly, SATB2 was positive in 16 of 18 cases (89%) with diffuse (3+ or 4+) positivity in 75% of the cases. The 2 CDH17-negative cases were positive for SATB2, and the 2 SATB2–negative cases were positive for CDH17. Nearly all CDH17-positive cases demonstrated a strong membranous staining, and the vast majority of SATB2-positive cases had a strong or intermediate nuclear staining.

Calretinin expression was observed in 12 of 18 cases (67%), with both nuclear and cytoplasmic staining. TFF3 was noted in 13 of 18 cases (72%), with cytoplasmic staining. The distribution seen in both calretinin and TFF3-positive cases was focal (1 to 2+), but the staining signal was strong in the majority of the cases. Focal (1+ or 2+) but strong cytoplasmic staining for MUC4 was seen in 13 of 18 cases (72%). Focal and weak cytoplasmic positivity for synaptophysin was noted in 3 cases (17%). Interestingly, 1 case (case 13) was diffusely and strongly positive for CK7 and negative for both CK20 and CDX2. This particular case presented with a large metastatic carcinoma in the left-side colon/mesentery 6 months after right-side colectomy. The histologic and immunostaining profile of the metastasis was identical to the primary tumor. Focal staining for p504S and villin was noted in a small percentage of cases. The detailed staining results for each case are summarized in Table 3.

Representative stains for case 16, cases 6 and case 15, and case 13 are shown in Figures 1, A through F; 2, A through F; and 3, A through D, respectively.

CDH17 Expression in 270 GI and Pancreatic Adenocarcinomas

CDH17 expression was seen in 123 of 125 cases (98%) of colorectal adenocarcinoma, with diffuse (3+ or 4+) and strong membranous staining in 94% of the cases. Twenty of 30 cases (67%) of esophageal adenocarcinoma were positive for CDH17, 2 cases with diffuse staining (3+). In contrast, positive staining for CDH17 was observed in only 25% and 18% of gastric and pancreatic adenocarcinomas, respectively. The detailed results are summarized in Table 4. Representative cases of adenocarcinoma of the colon, esophagus, and pancreas are shown in Figure 4, A through C.

CDH17 Expression in 1671 Non-GI/Nonpancreatic Tumors

The expression of CDH17 was observed in 55 of 1671 cases (3.3%) of non-GI and nonpancreatic tumors, including adenocarcinomas of the lung, endocervix, and endometrium, and rarely in hepatocellular carcinoma (HCC), prostatic adenocarcinomas, and urothelial carcinomas. Twenty-six of 198 lung adenocarcinomas were positive for CDH17, with diffuse staining (3+ or 4+) in 19% (5 of 26) of the cases. Fifteen of 55 cases of endocervical adenocarcinoma (27%) were positive for CDH17, with diffuse staining (3+ or 4+) in 40% (6 of 15) of the cases. The detailed staining results are summarized in Table 5. Representative cases of adenocarcinoma of the lung, endocervix, and endometrium are shown in Figure 4, D through F.

CDH17 Expression in 358 Cases of Normal Tissues

Colonic mucosa and duodenal mucosa were 100% positive for CDH17, with diffuse and strong staining in all cases. The mucosae of both gastric antrum and gastric

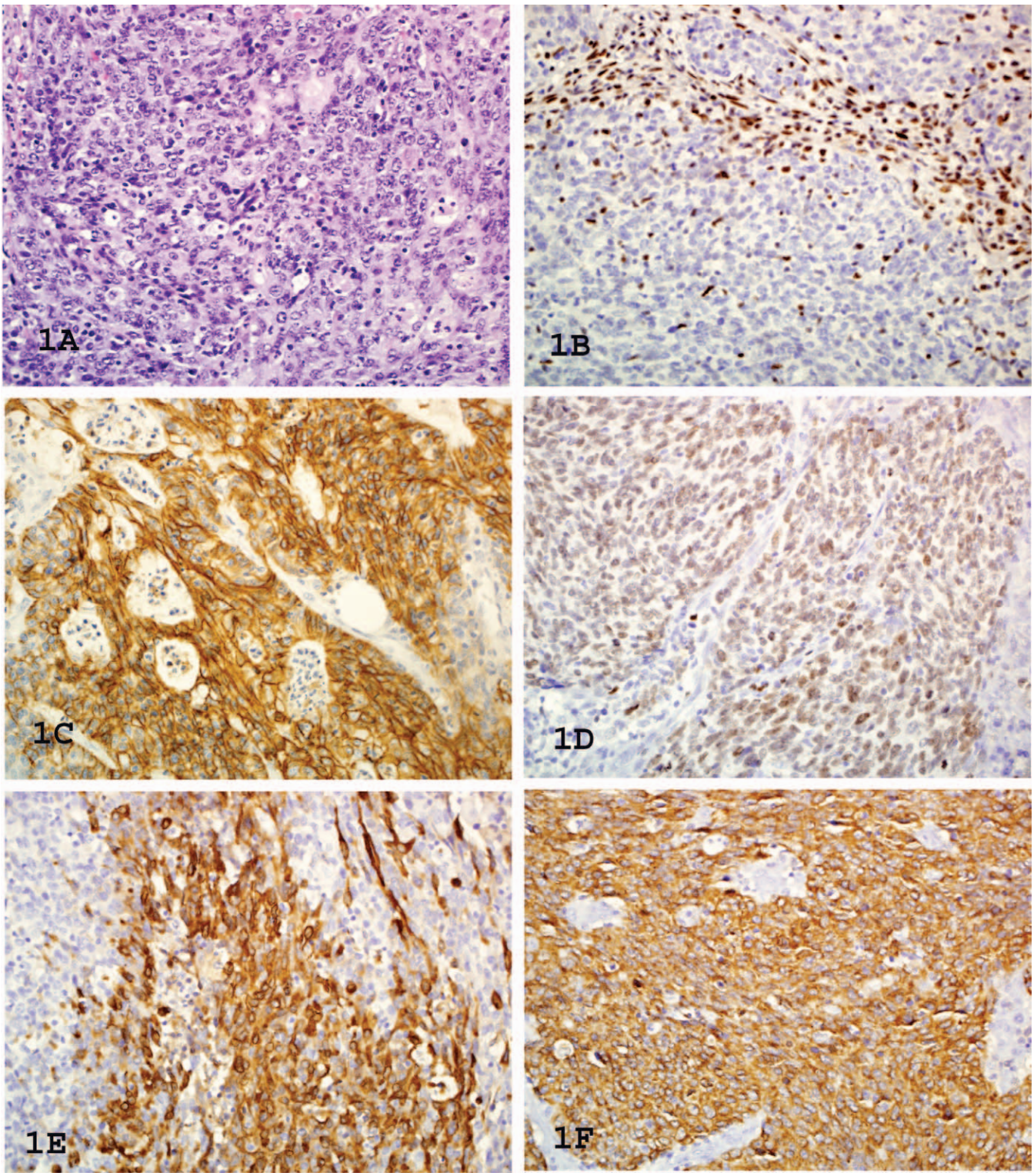

Figure 1. *A case of medullary carcinoma (case 16). Hematoxylin-eosin stain (A); loss of expression of MLH1 (B); positive for cadherin-17 (C), SATB-2 (D), TFF3 (E), and MUC4 (F) (original magnifications ×400).*

body were negative for CDH17. Hepatocytes were negative for CDH17, but 3 of 28 cases showed CDH17 positivity in bile ducts. Other normal tissues tested in this study were all negative for CDH17. The results are summarized in Table 6.

SATB2 Expression in 270 GI/Pancreatic Tumors

Expression of SATB2 was seen in 121 of 125 cases (96.8%) of colorectal adenocarcinoma, with diffuse (3+ or 4+) and strong nuclear staining in 92 cases (76%). Nineteen cases showed weak nuclear staining. In contrast,

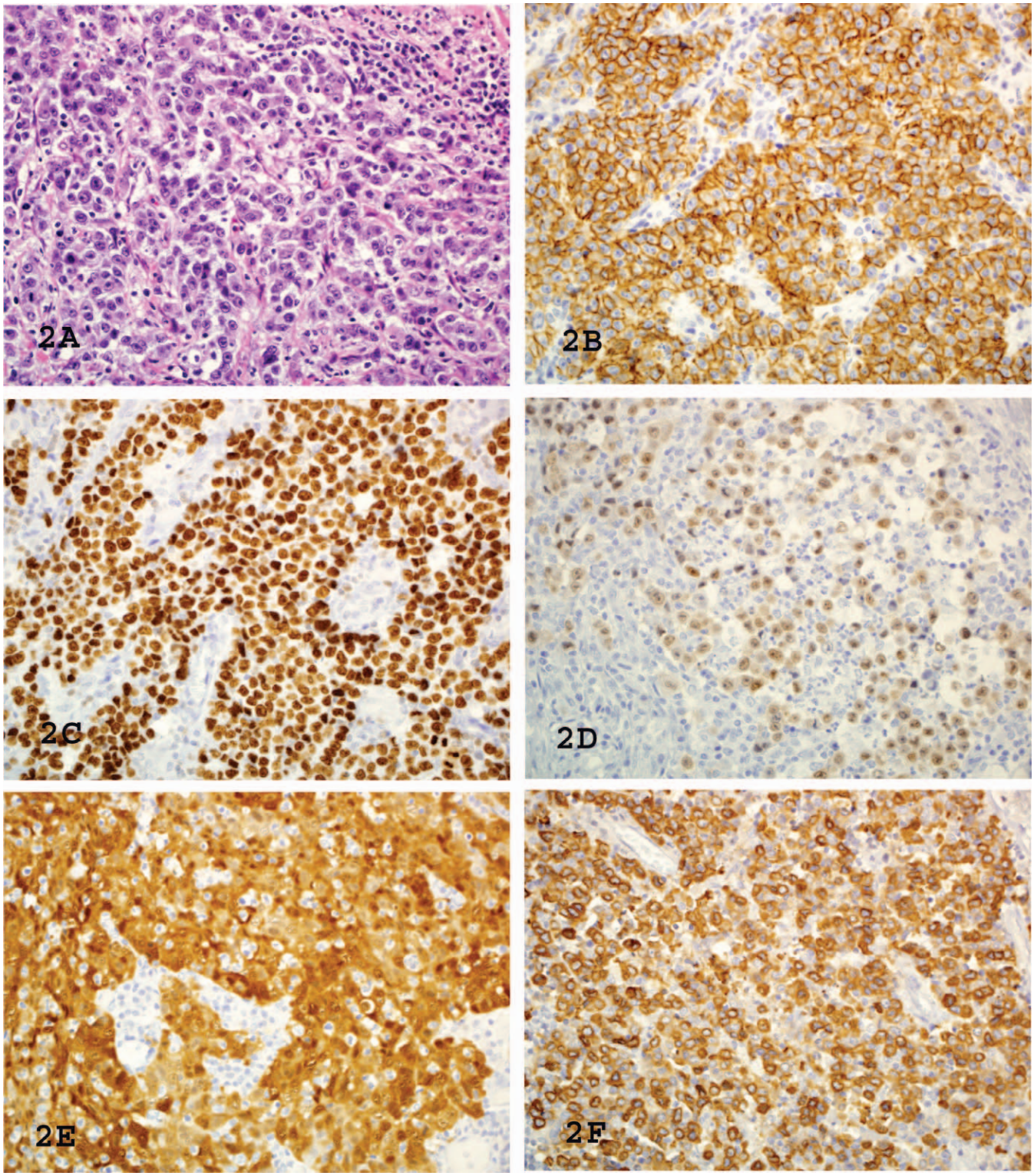

Figure 2. *A through D, A case of medullary carcinoma (case 6). Hematoxylin-eosin stain (A), strongly positive for cadherin-17 (B) and SATB-2 (C) and weakly positive for CDX2 (D). E and F, Another medullary carcinoma case (case 15) with strong staining for calretinin (E) and TFF3 (F) (original magnifications ×400).*

SATB2 expression was observed in only 6.7%, 0%, and 4.2% of adenocarcinomas of the esophagus, stomach, and pancreas, respectively. The staining signal was weak in these cases, except in one case of pancreatic adenocarcinoma with 4+ strong staining. The staining results are summarized in Table 7. Representative cases of adenocarcinomas of the colon and pancreas are shown in Figure 5, A and B.

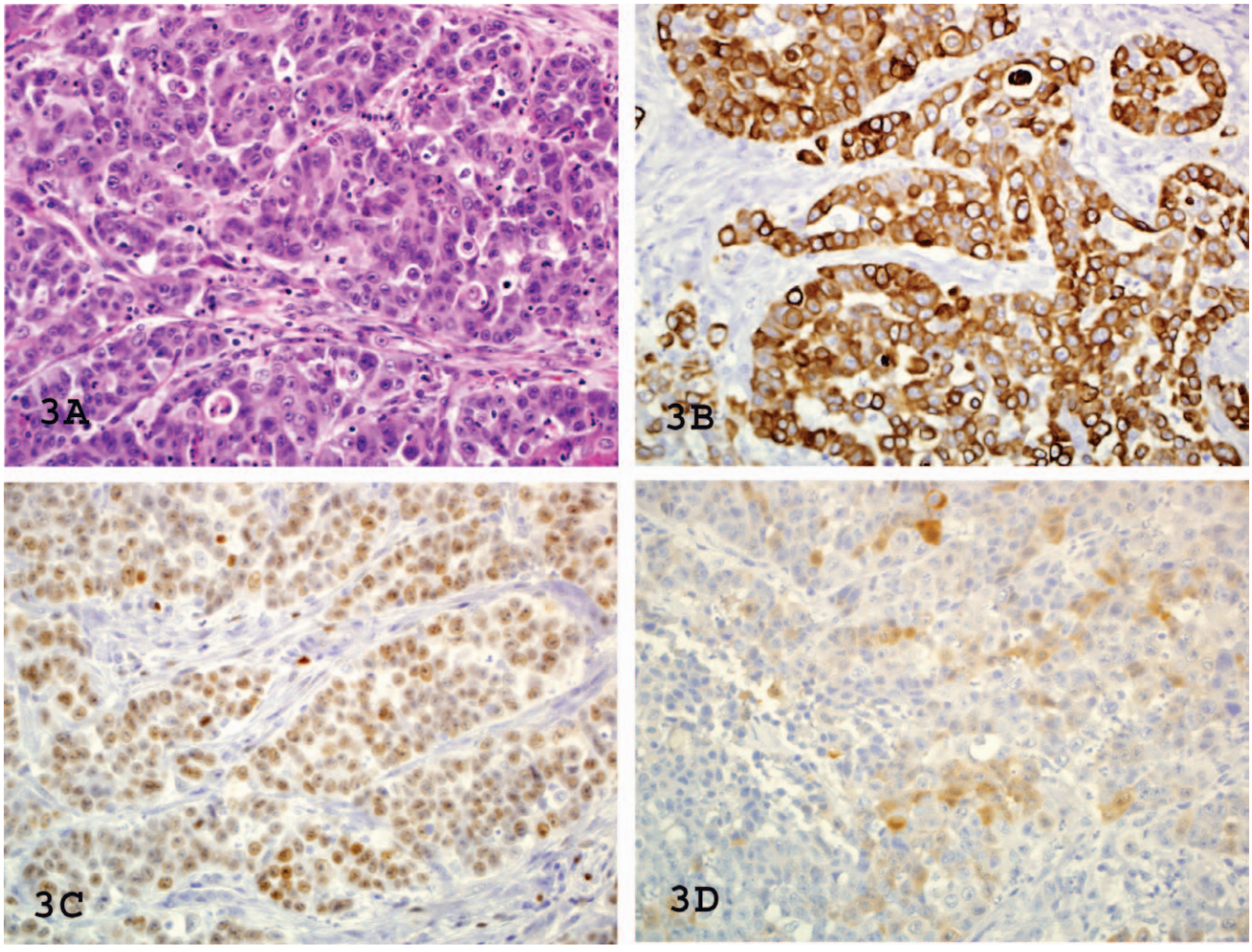

Figure 3. *A case of medullary carcinoma (case 13). Hematoxylin-eosin stain (A), positive for cytokeratin 7 (B), weakly positive for SATB-2 (C), focally positive for calretinin (D), and negative for cytokeratin 20 and for CDX2 (not shown) (original magnifications ×400).*

SATB2 Expression in 1671 Non-GI/Nonpancreatic Tumors

The expression of SATB2 was observed in 60 of 1671 cases (3.6%) of non-GI and nonpancreatic tumors, including mainly lung adenocarcinomas, lung squamous cell carcinomas, and bladder urothelial carcinomas, and rarely renal cell carcinomas, endometrial adenocarcinomas, and angiosarcomas. Thirteen of 121 pulmonary squamous cell carcinomas showed weak nuclear staining for SATB2, with diffuse staining (3+ or 4+) in only 3 cases. Ten of the 43 cases of invasive urothelial carcinoma (23%) were positive for SATB2, with diffuse (3+ or 4+) and strong staining in 2 cases. The remaining 8 cases showed focal and weak nuclear staining. The detailed immunostaining results are summarized in Table 5. Representative cases of urothelial carcinoma and angiosarcoma are shown in Figure 5, C and D.

SATB2 Expression in 358 Cases of Normal Tissues

All cases of colonic mucosa (100%) were positive for SATB2, with diffuse and strong staining. In contrast, duodenal mucosa and ileal mucosa were negative for SATB2 in all cases. The mucosae of both gastric antrum and gastric body were negative for SATB2. Bile ducts and benign

Table 4. Cadherin-17 (CDH17) Expression in 270 Gastrointestinal and Pancreatic Adenocarcinomas[a]

Diagnosis	Negative	1+	2+	3+	4+	No. Positive/ Total No. Cases (%)
Esophagus ADC (n = 30)	10	3	15	2	0	20/30 (67)
Stomach ADC (n = 20)	15	2	3	0	0	5/20 (25)
Colon ADC (n = 125)	2	0	7	8	108	123/125 (98)
Pancreas ADC (n = 95)	78	9	4	3	1	17/95 (18)

Abbreviation: ADC, adenocarcinoma.

[a] All positive cases show a strong or intermediate staining signal.

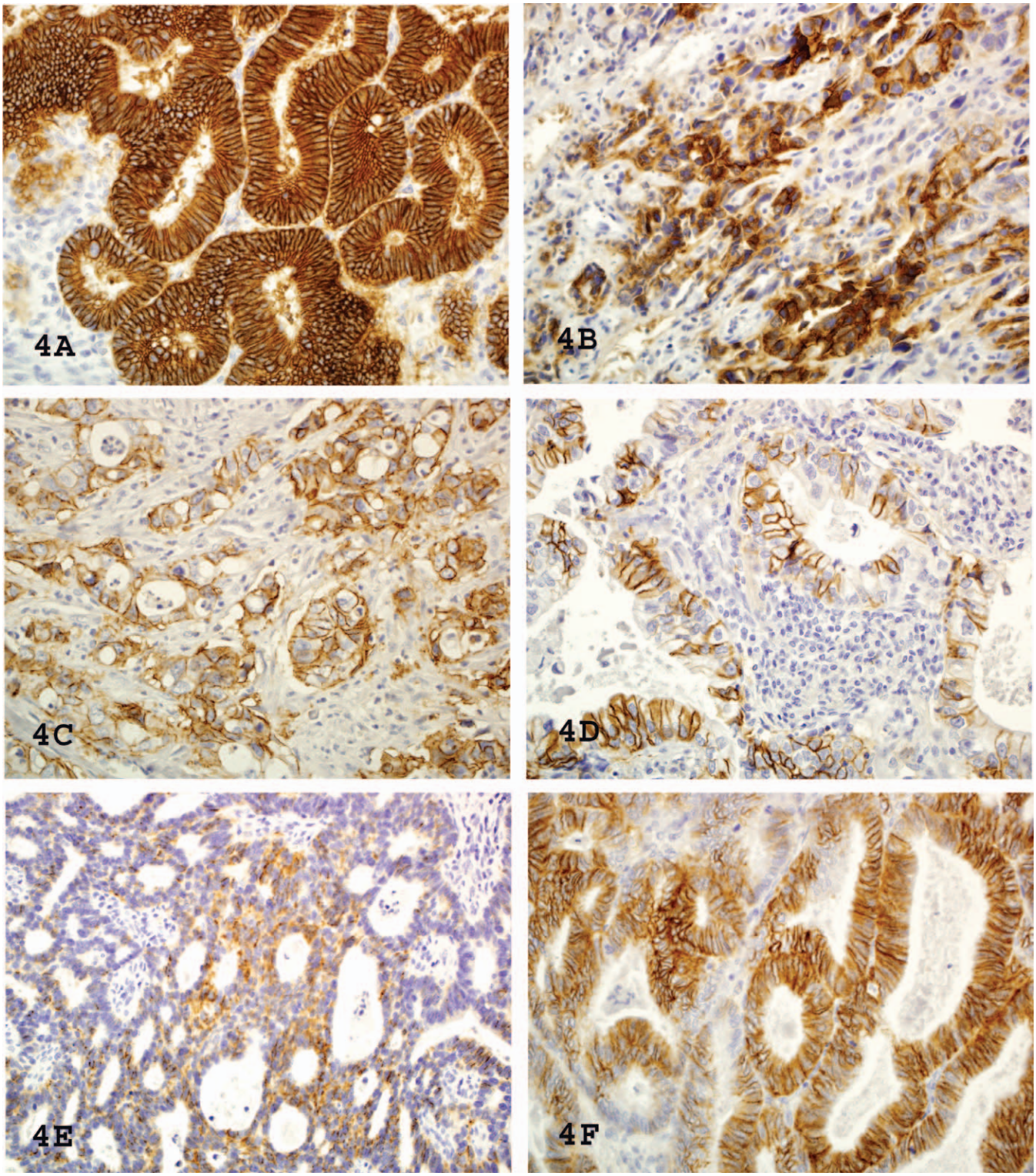

Figure 4. *Cadherin-17 membranous staining of adenocarcinoma of the colon (A), esophagus (B), pancreas (C), lung (D), endocervix (E), and endometrium (F) (original magnifications ×400).*

pancreatic ducts were negative for SATB2 as well. Three cases of seminal vesicles were focally (1+) and weakly positive for SATB2. Other normal tissues tested in this study were all negative for SATB2. The results are summarized in Table 6. Representative cases of normal colonic mucosa and normal small intestinal mucosa are shown in Figure 5, E and F.

COMMENT

Medullary carcinoma of the large intestine is a rare but distinct variant of colorectal adenocarcinoma. Our current study demonstrated that the incidence of MC was approximately 1.6% (18 of 1144 consecutive cases of colorectal adenocarcinoma). Clinical characteristics of these cases in

Table 5. Expression of Cadherin-17 (CDH17) and SATB2 in 1671 Nongastrointestinal Tumors

Diagnosis	CDH17-Positive Cases, No. (%)	SATB2–positive cases, No. (%)
Lung adenocarcinoma (n = 198)	26 (13)	6 (3)[a]
Lung squamous cell carcinoma (n = 121)	0	13 (10.7)
Lung mesothelioma (n = 18)	0	0
Breast ductal carcinoma (n = 72)	0	0
Breast lobular carcinoma (n = 48)	0	0
Clear cell RCC, low grade (n = 34)	0	0
Clear cell RCC, high grade (n = 38)	0	3W (7.9)
Chromophobe RCC (n = 15)	0	0
Oncocytoma (n = 15)	0	0
Papillary RCC (n = 16)	0	3W (18.8)
Endocervical ADC, invasive (n = 55)	15 (27)	5 (9)
Endocervical ADC, in situ (n = 16)	5 (31)	0
Endometrial ADC, FIGO I (n = 38)	1 (3)	1 (3)
Endometrial ADC, FIGO II (n = 55)	4 (8)	2 (3.6)
Endometrial ADC, FIGO III (n = 38)	0	2 (5.3)
Bladder invasive UC (n = 43)	3 (7)	10 (23)
Bladder noninvasive UC (n = 38)	0	7 (18.4)
Ovarian serous carcinoma (n = 41)	0	1 (2.1)
Hepatocellular carcinoma (n = 18)	1 (6)	0
Prostate ADC, low grade (n = 97)	0	0
Prostate ADC, high grade (n = 35)	1 (3)	0
Thyroid papillary carcinoma (n = 47)	0	0
Thyroid follicular carcinoma (n = 37)	0	0
Thyroid follicular adenoma (n = 51)	0	0
Thyroid MC (n = 12)	0	0
Melanoma (n = 93)	0	0
Germ cell tumors (n = 60)	0	0
Pheochromocytoma (n = 13)	0	0
Adrenal adenoma (n = 23)	0	0
Lymphoma (n = 84)	0	0
Myeloma (n = 80)	0	0
Angiosarcoma (n = 35)	0	3 (8.6)[b]
Hemangioma (n = 51)	0	0
Gastrointestinal stromal tumor (n = 36)	0	0
Total positive cases, No. (%)	**55** (3.3)	**60** (3.6)

Abbreviations: ADC, adenocarcinoma; FIGO, International Federation of Gynecology and Obstetrics; MC, medullary carcinoma; RCC, renal cell carcinoma; UC, urothelial carcinoma; W, weak staining.

[a] 3 of 6 positive cases showed weak staining.
[b] 2 of 3 positive cases showed weak staining.

Table 6. Expression of Cadherin-17 (CDH17) and SATB2 in 358 Cases of Normal Tissues

Tissue/Organ	Total No. Cases	CDH17-Positive Cases, No. (%)	SATB2–Positive Cases, No. (%)
Stomach, antrum	20	0	0
Stomach, body	20	0	0
Duodenum	20	20 (100)	0
Ileum	20	20	0
Colon	100	100 (100)	100 (100)
Liver (hepatocytes)	28	0[a]	0
Lung	20	0	0
Prostate	24	0	0
Ovary	21	0	0
Thyroid	15	0	0
Kidney	20	0	0
Uterine cervix	27	0	0
Seminal vesicles	23	0	3 (13)[b]

[a] Hepatocytes were negative for CDH17, but in 3 cases, the bile ducts were focally positive for CDH17.
[b] Focal, weak positivity for SATB2 in 3 cases.

the current study were very similar to those reported in the literature, in which a typical case presented in an elderly female with a large tumor in the right side of the colon.[1–9]

More than 80% of MC cases in this study demonstrated evidence of MSI by loss of MLH1 and PMS2, with the exception of one case that showed loss of MSH2 and MSH6. No additional information on this case was available in regards to the possibility of hereditary nonpolyposis colorectal cancer. These data were similar to reports in the literature.[8,9,16] Importantly, reduced or absent expression of CK20, which has been known to be associated with the finding of MSI status, was seen in 75% of cases.[9] There is no strong correlation between MSI and decreased CDX2 expression.[9] However, decreased CDX2 expression is also a frequent finding in MC.[9,18] Expression of CDX2 was noted in 19% of the MC cases reported by Winn et al.[16] In the current study, CDX2 expression was seen in 67% (12 of 18) of MC cases. This percentage appeared to be much higher than that reported by Winn et al,[16] which may be attributed to using large routine tissue sections and counting focal and weak staining signal in the current study. However, only 5 of 18 cases (27%) demonstrated diffuse (3+ or 4+) and intense staining (strong or intermediate). The remaining positive cases showed only a focal and weak staining signal, which has limited utility in daily practice because a pathologist rarely interprets focal and weak staining as a true-positive result unless dealing with a predictive biomarker such as estrogen receptor or progesterone receptor.

Similar to the previous study by Winn et al,[16] our current study further confirmed their findings of the expression of calretinin and TFF3 in a significant percentage of MC cases. The positive staining for both calretinin and TFF3 tended to be focal (1+ or 2+) but very strong and easily seen at a low magnification. Therefore, both markers can be used as potential diagnostic markers. Another intestinal differentiation marker, MUC4, tested in this study was shown to be a useful marker as well; it was expressed in 72% of MC cases, with a focal but very intense staining signal. Two other markers, P504S and villin, demonstrated only focal and weak staining in a minority of cases. Expression of the neuroendocrine marker synaptophysin was observed in 17% of MC cases, with only focal staining (1+), which was in agreement with the report by Wick et al.[20]

Given the undifferentiated morphology and reduced or absent expression of both CK20 and CDX2 in these tumors, a diagnostic challenge can be encountered, especially when working on a tumor of unknown primary. To complicate this matter further, rare MC cases, such as case 13 in this study, can be positive for CK7 and negative for both CK20 and CDX2. Therefore, searching for more sensitive and specific markers is imperative. The 2 recently described immunomarkers CDH17 and SATB2 may potentially serve this purpose. In the current study, 89% of MC cases were positive for CDH17 and SATB2, respectively. Importantly, 2 CDH17-negative cases were both positive for SATB2, and 2 SATB2–negative cases were positive for CDH17. Therefore, CDH17 and SATB2 are complementary and, when used together, could identify all MC cases in this study. Taking these results together, a typical MC case has an immunohistochemical staining profile of $CK20^{-}/CDX2^{-}$ or focally +/$MLH1^{-}/PMS2^{-}/CDH17^{+}/SATB2^{+}$/calretinin focally +/MUC4 focally +/TFF3 focally +.

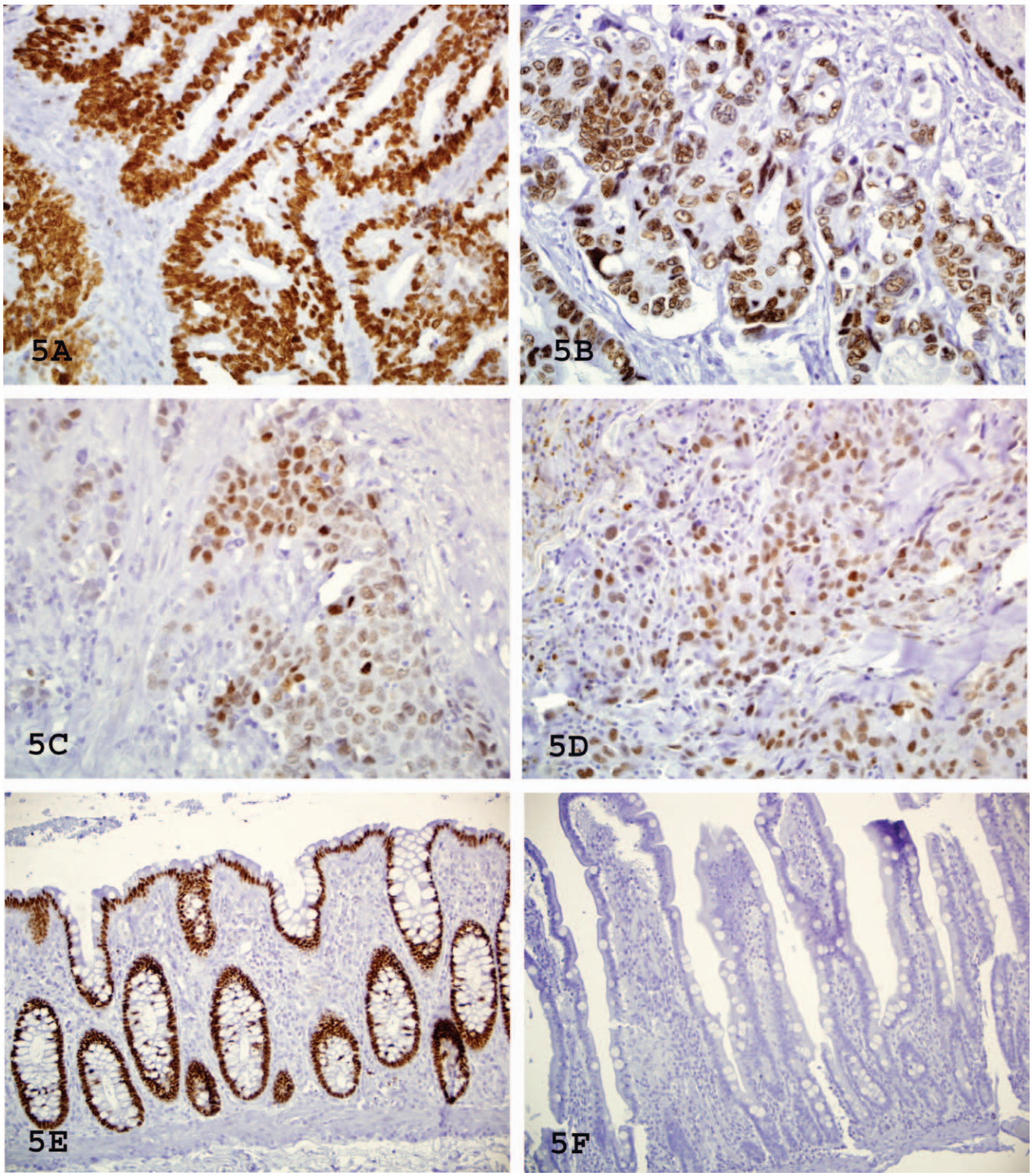

Figure 5. *SATB2 nuclear staining of colorectal adenocarcinoma (A), pancreatic adenocarcinoma (B), urothelial carcinoma (C), angiosarcoma (D), and normal colon (E). Note that normal small intestinal mucosa is negative for SATB2 (F) (original magnifications ×400).*

In addition to their utility in the diagnosis of MC of the large intestine, we also investigated CDH17 and SABT-2 expression in a large number of tumors and normal tissues from various organs. Our results indicated that SATB2 was a highly sensitive and specific marker for adenocarcinomas of the colon and rectum, with a diagnostic sensitivity of 97% (121 of 125 cases) in colorectal carcinomas. Twelve of 125 colorectal carcinomas were poorly differentiated. All 4 SATB2–negative colorectal carcinomas (3 were poorly differentiated carcinomas) were positive for CDH17. In this study, SATB2 expression in MC was lower than that in non-MC colorectal adenocarcinomas (89% versus 97%), which

Table 7. SATB2 Expression in 270 Cases of Gastrointestinal and Pancreatic Adenocarcinoma						
Diagnosis	Negative	1+	2+	3+	4+	Positive Cases, No. (%)
Esophagus ADC (n = 30)	28	2W	0	0	0	2 (6.7)
Stomach ADC (n = 20)	21	0	0	0	0	0
Colon ADC (n = 125)	4	5/1W	7/9W	10/5W	84	121 (96.8)
Pancreas ADC (n = 95)	91	1	1/1W	0	1	4 (4.2)

Abbreviations: ADC, adenocarcinoma; W, weak staining.

was compatible with the observation of the down-regulation of SATB2 protein expression in poorly differentiated and metastatic colorectal adenocarcinomas.[26,27] Only rare pancreatic adenocarcinomas and no adenocarcinomas of the esophagus and stomach were positive for SATB2, which was similar to the report by Magnusson et al.[27] We also confirmed that a small percentage of tumors, such as lung adenocarcinomas, lung squamous cell carcinomas, and bladder urothelial carcinomas, can be focally and weakly positive for SATB2, and found only rare cases of adenocarcinomas from the endometrium, ovary, and endocervix to be positive for SATB2. Magnusson et al[27] also reported that sinonasal carcinomas can be positive for SATB2, CK20, and CDX2. More recently, SATB2 expression was reported as a novel marker of osteoblastic differentiation of bone and soft tissue tumors.[30] Hence, caution should be taken, because there is little value in using SATB2 to differentiate a sarcomatoid carcinoma with bone metastasis from an osteogenic sarcoma. Additionally, expression of SATB2 was also noted in lymphoid cells in this study; one should be aware of this potential pitfall when interpreting a tumor with dense lymphoid infiltration such as MC. Preliminary data from this study also show that SATB2 was not expressed in large B-cell lymphomas. It is uncertain whether loss of SATB2 expression may play a role in the malignant transformation of lymphoid cells.

Furthermore, our unpublished data demonstrate that SATB2 was expressed in a majority of neuroendocrine tumors of the colon, rectum, and appendix but rarely in neuroendocrine tumors from the stomach, duodenum, small intestine, pancreas, and lung. Therefore, SATB2 can potentially be used as a specific marker to identify a neuroendocrine tumor of the lower GI tract. Li and coworkers[31] reported similar findings. Another interesting observation in this study is the lack of expression of SATB2 in normal duodenum and small intestine. In addition, the preliminary study on 4 cases of primary small bowel adenocarcinoma showed that 2 cases were negative for SATB2 and 2 cases showed focal and weak staining. As known, adenocarcinomas of the small intestine may share a similar immunophenotype with colorectal carcinomas,[32] so differentiation between a small intestinal adenocarcinoma and a colorectal adenocarcinoma may not be straightforward, even though a significant percentage of adenocarcinomas of the small intestine can express CK7.[32] These preliminary data suggested that adenocarcinoma of the small intestine is likely to be negative or only focally positive for SATB2, which, in turn, can be used as a potential marker to distinguish an adenocarcinoma of the small intestine from a colorectal adenocarcinoma. Further investigation would be needed to support this initial finding.

In contrast to SATB2, CDH17 was expressed in 98.4% (123 of 125) of cases of colorectal adenocarcinomas and also in a significant percentage of adenocarcinomas of the esophagus, stomach, and pancreas. The 2 CDH17-negative colorectal carcinomas (both were poorly differentiated carcinomas) were weakly positive for SATB2. Combining the CDH17 and SATB2 staining results, 100% of colorectal carcinomas in this study, including 18 MC cases and 12 poorly differentiated colorectal carcinomas on tissue microarray sections, were positive for either or both of these 2 markers. Our current study demonstrated similar staining results for CDH17 expression in colorectal adenocarcinomas to those reported by Panarelli et al.[25] However, in our study, CDH17 expression in adenocarcinomas of the pancreas, stomach, and esophagus was lower than in their report.[25] We repeated the CDH17 immunostain and obtained identical results. In our study, when less than 5% of the tumor cells were stained, the case was interpreted as negative. In the study by Panarelli et al, [25] the criterion for rendering a negative staining was not clearly stated. If any staining of tumor cells was regarded as a positive case, then the sensitivity would have been higher in their study when compared with the 5% cutoff criterion we used in this study. No satisfactory explanation can be given at this point.

In summary, these data (1) support and confirm the previously reported clinicopathologic features of MC presenting as a large tumor in the right side of the colon in an elderly woman, with frequent loss of both MLH1 and PMS2; (2) substantiate the reports of the loss or marked reduction of expression of CK20 and CDX2 and expression of calretinin and TFF3 in the majority of MC cases; (3) provide evidence for CDH17 and SATB2 being the most sensitive and specific markers for the diagnosis of MC of the large intestine; and (4) further refine the diagnostic sensitivity and specificity of both CDH17 and SATB2 by broadening the testing in a large number of tumors and normal tissues from various organs. Based on this study and the review of the literature, we propose including MLH1, CDH17, and SATB2 in a routine diagnostic immunohistochemical panel when working on a tumor of unknown primary, especially in an elderly patient with a $CK7^{-}/CK20^{-}$ carcinoma.

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