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Practical Immunohistochemistry in Neoplastic Pathology of the Gastrointestinal Tract, Liver, Biliary Tract, and Pancreas

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• ***Context.***—**Immunomarkers with diagnostic, therapeutic, or prognostic values have been increasingly used to maximize the benefits of clinical management of patients with neoplastic diseases of the gastrointestinal tract, liver, biliary tract, and pancreas.**

Objectives.—**To review the characteristics of immunomarkers that are commonly used in surgical pathology practice for neoplasms of the gastrointestinal tract, liver, biliary tract, and pancreas, and to summarize the clinical usefulness of immunomarkers that have been discovered in recent years in these fields.**

Data Sources.—**Data sources include literature review, authors' research data, and personal practice experience.**

Conclusions.—**Immunohistochemistry is an indispensable tool for the accurate diagnosis of neoplastic diseases of the gastrointestinal tract, liver, biliary tract, and pancreas. Useful immunomarkers are available to help distinguish malignant neoplasms from benign conditions, determine organ origins, and subclassify neoplasms that are morphologically and biologically heterogeneous. Specific immunomarkers are also available to help guide patient treatment and assess disease aggressiveness, which are keys to the success of personalized medicine. Pathologists will continue to play a critical role in the discovery, validation, and application of new biomarkers, which will ultimately improve patient care.**

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Malignant neoplasms of the gastrointestinal (GI) tract, liver, biliary tract, and pancreas are the most common cancers of the body, with an estimated 304 930 new cases and an estimated 153 030 deaths in the year 2016 in the United States.[1] Most of these neoplasms are aggressive malignancies with high mortality rates. In 2012, cancers of the colorectum and pancreas were the third and fourth leading cancer deaths, respectively, in both male and female patients. Liver cancer was also the fifth leading cancer death in male patients. The prognosis of pancreatic and hepatobiliary cancers is extremely poor, with adjusted 5-year relative survival rates of 8% and 18%, respectively. In fact, the death rates are increasing for cancers of the liver and pancreas despite a significant drop of 23% in the overall cancer death rate since 1991.[1] One of the major obstacles in improving patient survival for these cancers is the failure of early diagnosis.

Despite the rapid advances in molecular and genetic testing in the past decade, immunohistochemistry remains a major ancillary tool for pathologists to make critical contributions to patient care. This is particularly true when immunomarkers can be used to help make the distinction between malignant and benign conditions so that an accurate diagnosis can be made on small biopsies obtained from lesions that are detected at early stages with advanced imaging and endoscopic technologies. Immunomarkers are also available to help predict the biologic behavior of diseases to guide treatment and surveillance decisions, which are also critically important to the success of patient survival.

In this article, we attempt to provide a comprehensive review on immunomarkers that are commonly used in surgical pathology practice for neoplasms of the digestive system, and to summarize the clinical usefulness of immunomarkers that have been discovered in recent years in these fields. Given the similarity of the topics, the content of this article may have some overlap with that of several review articles that were published recently in this journal.[2–4]

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Table 1. Commonly Used Diagnostic Immunomarkers for Adenocarcinomas of the Digestive System					
Adenocarcinoma	**CK20, %**	**CDX2, %**	**SATB2, %**	**CK7, %**	**CDH17, %**
Colorectal	90	95	90	18	98
Small intestinal	60	70	46[a]	60	ND
Gastric	45	55	0	80	60
Esophageal	40	45	7	85	75
Pancreaticobiliary	35	20	5	95	30

Abbreviations: CK, cytokeratin; ND, not done.

[a] Patchy and weak staining in most cases; strong and diffuse staining seen in <10% of cases.

IMMUNOMARKERS FOR ADENOCARCINOMAS OF THE GI TRACT

General Immunophenotypes

The most commonly used immunomarkers for adenocarcinomas of the GI tract are cytokeratin 7 (CK7), CK20, and CDX2. Special AT-rich sequence-binding protein 2 (SATB2) and cadherin-17 (CDH17) are new markers that have been increasingly used recently (Table 1). Mucins (MUCs) appear to have limited utility in the workup of carcinomas of the digestive system.

CK7 and CK20.—The differential expression patterns of CK7 and CK20 are routinely used in the workup for adenocarcinomas of unknown primary. The $CK7^{-}/CK20^{+}$ phenotype is relatively restricted to colorectal adenocarcinoma (CRC) and appears to be more specific than CDX2.[5,6] In a recent study, the $CK7^{-}/CK20^{+}$ phenotype showed a specificity of 98% to 99% for CRC.[7] However, the sensitivity of the $CK7^{-}/CK20^{+}$ phenotype in CRC is lower than that of CDX2, because at least 10% of CRCs also express CK7.[8] The frequency of CK7 expression in rectal adenocarcinomas can be much higher.[9] In addition, CK20 expression may be lost in a significant subset of CRCs of the right colon,[10] and in most cases of colorectal medullary carcinoma.[11] In CRC, CK20 positivity is typically diffuse, whereas CK7 immunoreactivity, if present, is often focal or patchy. CK7 expression in CRC may also be less intense than that seen in non-CRC adenocarcinomas that typically express CK7.[10,12,13]

Other predominantly $CK20^{+}$ cancers include adenocarcinomas of the appendix, adenocarcinomas of the urachus, and a subset of adenocarcinomas of the small intestine and urinary bladder. Merkel cell carcinoma and small cell carcinoma of the salivary glands are also $CK7^{-}/CK20^{+}$, with a paranuclear dotlike CK20 staining pattern.[14]

Most gastroesophageal and pancreaticobiliary adenocarcinomas are $CK7^{+}/CK20^{-}$, but a small fraction of cases are $CK7^{+}/CK20^{+}$ or even $CK7^{-}/CK20^{+}$.[5,15] CK20 expression in these tumors is typically focal, if present, which is in contrast to that seen for CRC. Using the CK7/CK20 staining pattern as well as other immunomarkers to separate adenocarcinomas of the gastroesophageal junction into origins of the distal esophagus and proximal stomach is difficult in practice because of substantial immunophenotypic overlap. Adenocarcinoma of the anal glands, which may be confused with rectal adenocarcinoma histologically, is also typically $CK7^{+}/CK20^{-}$. This tumor is also negative for CDX2 and may show loss of expression of both p63 and CK5/6 proteins.[16]

CDX2.—CDX2 is a homeobox transcription factor essential for the maintenance of intestinal phenotype and is a highly sensitive immunomarker for CRC. However, its specificity for CRC is low because its expression may also be detected in a variety of adenocarcinomas, such as those of the upper GI tract, pancreaticobiliary tract, urinary bladder, uterus, ovary, and lung.[6,17–22] Enteric-type sinonasal adenocarcinomas consistently express enteric immunomarkers CDX2, CK20, and villin.[23,24] Mucinous adenocarcinomas of the lung frequently express CDX2 and CK20, with loss of expression of TTF-1 and napsin A.[25] Studies have shown a greater intratumoral heterogeneity of CDX2 expression in extraintestinal adenocarcinomas compared with the strong and diffuse staining pattern observed for CRC.[12] In general, CDX2-expressing adenocarcinomas are of intestinal type histologically or show enteric differentiation, as seen in the stomach.[5] Loss of expression of CDX2 and CK20 occurs in 15% to 20% of CRCs with microsatellite instability,[26–28] and is more frequently seen in poorly differentiated or undifferentiated carcinomas, such as medullary carcinoma.[11,28–30] *BRAF*-mutated, microsatellite-stable CRCs may also have reduced CDX2 expression accompanied by increased CK7 expression.[31] These tumors have been shown to behave more aggressively, with frequent lymph node metastasis and worse patient survival compared with garden variety CRCs. Loss of CDX2 expression may also help identify a high-risk subgroup of patients with stage II CRCs who may benefit from adjuvant chemotherapy.[32]

MUC Proteins.—Mucins, or mucus glycoproteins, are widely expressed in normal epithelial cells and their derived neoplasms. Their diagnostic and prognostic significance has been extensively studied in GI and pancreaticobiliary neoplasms. In general, MUC1 is preferentially expressed in pancreaticobiliary adenocarcinomas, whereas MUC2 is preferentially expressed in intestinal-type adenocarcinomas, such as CRC.[33,34] However, there is substantial immunophenotypic overlap among various carcinomas, with a wide range of staining heterogeneity, which markedly limits their diagnostic value in the workup for carcinomas of unknown primary.[35–37]

SATB2.—SATB2 is a nuclear matrix–associated transcriptional regulator that is involved in a wide spectrum of biologic functions, such as neuron specification, osteoblastic differentiation, skeletal development, and immunoglobulin μ gene expression.[38–41] By immunohistochemistry, SATB2 is expressed in normal tissues in a tissue-specific manner, with strong nuclear staining restricted to epithelial cells lining the colorectum and appendix, and a subset of neurons in the brain. Weak to moderate immunoreactivity is also observed in some lymphocytes and epithelial cells lining the seminal vesicles, seminiferous ducts, and epididymis.[11,42] SATB2 expression is not detected in epithelial cells lining the normal small intestine, stomach, or pancreaticobiliary tract. Studies have shown that SATB2 is a relatively sensitive and specific marker for CRC, with retained expression in most (85%–97%) primary and metastatic CRCs. In non-CRC digestive system cancers, SATB2 expression was seen in 0% of gastric, 6.7% of esophageal, 0% to 4.2% of pancreatic, and 6.7% of biliary adenocarcinomas.[11,42]

As a diagnostic marker for CRC, SATB2 offers similar to slightly lower sensitivity to traditional markers CDX2 and CK20 but shows promising utility because of its relatively high specificity.[42] Recently, Dragomir et al[7] demonstrated that when SATB2 was added, the positive predictive power for CRC improved from 93% to 99% when a tumor was $CK7^{-}/CK20^{+}$. SATB2 can also label poorly differentiated or undifferentiated CRCs, which may lack the expression of conventional intestinal markers CK20 and CDX2. In the study by Lin et al,[11] SATB2 and CDH17 were each expressed in 16 of 18 medullary carcinomas of the colon (89%), although most of these cases did not express CK20 and CDX2. Interestingly, the 2 $CDH17^{-}$ cases were both positive for SATB2, and vice versa. Nearly all positive cases showed a strong and diffuse staining pattern for SATB2.

Most studies have noted the diffuse and strong staining pattern to be highly characteristic of, although not entirely exclusive to, CRC (Figure 1, A). On the contrary, focal and/or weak SATB2 expression appears to be much less specific. In the study by Dragomir et al,[7] 102 of 458 non-CRC tumors (22%) showed detectable SATB2 immunoreactivity, with most showing scant positive cells. Only 16 non-CRC cases (3.5%) exhibited nuclear staining in more than 75% of tumor cells. These cases included neuroendocrine tumors, renal/urothelial cancers, Merkel cell carcinomas, tumors of the small intestine, lung cancer, and gynecologic cancer. In the study by Lin et al,[11] 60 of 1671 non-CRC cases (3.6%) showed SATB2 expression. Diffuse positivity was noted in only 3 of 121 pulmonary squamous cell carcinomas and 2 of 43 urothelial carcinomas. Of 145 adenocarcinomas from the esophagus, stomach, and pancreas, only 1 case from the pancreas showed the diffuse and strong staining pattern.

It is interesting to note that SATB2 is also a highly sensitive biomarker for osteoblastic differentiation in benign and malignant mesenchymal tumors, such as osteosarcoma.[43,44] The other type of tumor that shows a high frequency of SATB2 expression is sinonasal carcinoma, seen in 5 of 9 cases (56%) in 1 study.[42]

CDH17.—CDH17 is a member of the cadherin superfamily but is distinguished from classic cadherins by its unique structural and functional features.[45,46] Also known as liver-intestine cadherin, CDH17 was initially discovered to be a novel calcium-dependent cell adhesion molecule expressed in the liver and intestine of rats.[45,47] In humans the distribution of CDH17 is essentially limited to epithelial cells lining the small and large intestines.[11,46,48] Epithelial cells of the esophagus and stomach as well as hepatocytes are $CDH17^{-}$. Only occasionally is its immunoreactivity detected in epithelial cells lining the intrahepatic bile ducts and small pancreatic ducts.[11,48]

A few studies have demonstrated the clinical utility of CDH17 as an immunomarker of GI adenocarcinomas.[11,48–50] CDH17 is highly sensitive for primary and metastatic CRCs and is positive in up to 96% to 99% of examined cases. However, CDH17 positivity is also seen at a high frequency in adenocarcinomas of the esophagus (67%–82%), stomach (25%–90%), pancreas (18%–52%), and bile duct (27%–53%).[11,48,50] Interestingly, CDH17 labels adenocarcinomas of the digestive system with higher sensitivity and specificity than CDX2, although CDH17 is thought to be transcriptionally regulated by CDX2. Like SATB2, CDH17 can be positive in CRCs with poorly differentiated or undifferentiated morphology, such as medullary carcinoma.[11] Furthermore, CDH17 appears to show a lower frequency of expression in nondigestive tumors than CDX2. The available data have shown that CDH17 expression can be detected at a low frequency in adenocarcinomas of the endocervix, endometrium, and lung, and rarely in hepatocellular carcinoma and prostatic adenocarcinoma.[11,48]

Distinction Between CRC and Adenocarcinoma of the Urinary Bladder

The distinction between primary adenocarcinoma of the urinary bladder and CRC secondarily involving the bladder is a known diagnostic challenge because of their morphologic similarities. A number of immunomarkers have been tested in this regard, which include CK7, CK20, CDX2, villin, thrombomodulin, α-methylacyl coenzyme-A racemase, carcinoembryonic antigen (CEA), GATA3, p63, CDH17, β-catenin, and many others.[51–56] Among all tested markers, β-catenin is the only one that can be reliably used to aid in the distinction. Specifically, nuclear β-catenin expression is detected in more than 80% of CRCs but has never been seen in primary adenocarcinoma of the bladder.[51,55,56] Instead, bladder adenocarcinomas show only membranous staining. Thus, membranous β-catenin staining will not help, but nuclear staining indicates a colorectal origin. Our data also demonstrate SATB2 expression in 46% of primary adenocarcinomas of the bladder (H.L.W., unpublished data, 2016), which limits its utility in the distinction.

Distinction Between CRC and Adenocarcinoma of the Small Intestine

Primary adenocarcinoma of the small intestine is morphologically similar to or indistinguishable from CRC, but it differs from CRC tumorigenetically.[57] Immunohistochemically, small intestinal adenocarcinomas more frequently express CK7, and less frequently express CK20, CDX2, and α-methylacyl coenzyme-A racemase, in comparison with CRCs.[58–62] Interestingly, a $CK7^{+}/CK20^{-}$ pattern is much more commonly seen in Crohn-associated small intestinal adenocarcinomas than in sporadic cases.[62,63]

Although SATB2 is not expressed in normal small intestinal epithelium, it was expressed in 46% of small intestinal adenocarcinomas we examined.[62] However, SATB2 expression is typically patchy and weak in these tumors (Figure 1, B). Less than 10% of small intestinal adenocarcinomas exhibit a strong and diffuse staining pattern, in contrast to CRCs, where the strong and diffuse staining pattern is observed in 76% of cases.

Distinction Between Appendiceal and Ovarian Neoplasms

Recent studies have shown that SATB2 is of diagnostic value in distinguishing primary ovarian tumors from appendiceal metastasis. SATB2 expression is virtually absent in primary ovarian mucinous tumors (Figure 2, A and B) unless a component of mature teratoma is present.[64–66] In contrast, SATB2 is frequently expressed in low-grade appendiceal mucinous neoplasms (Figure 2, C and D). SATB2 also appears to be a reliable marker that can be used to distinguish metastatic adenocarcinoma of appendiceal origin from primary ovarian adenocarcinoma. In the study by Moh et al,[64] for example, SATB2 expression was observed in 8 of 10 low-grade appendiceal mucinous neoplasms and 4 of 4 high-grade appendiceal adenocarcinomas that metastasized to the ovaries. In primary ovarian tumors, SATB2 was detected in 0 of 22 mucinous cystadenomas, 4 of 12 mucinous cystadenomas with mature teratomatous components (33%), 1 of 60 mucinous borderline tumors (1.6%), 0

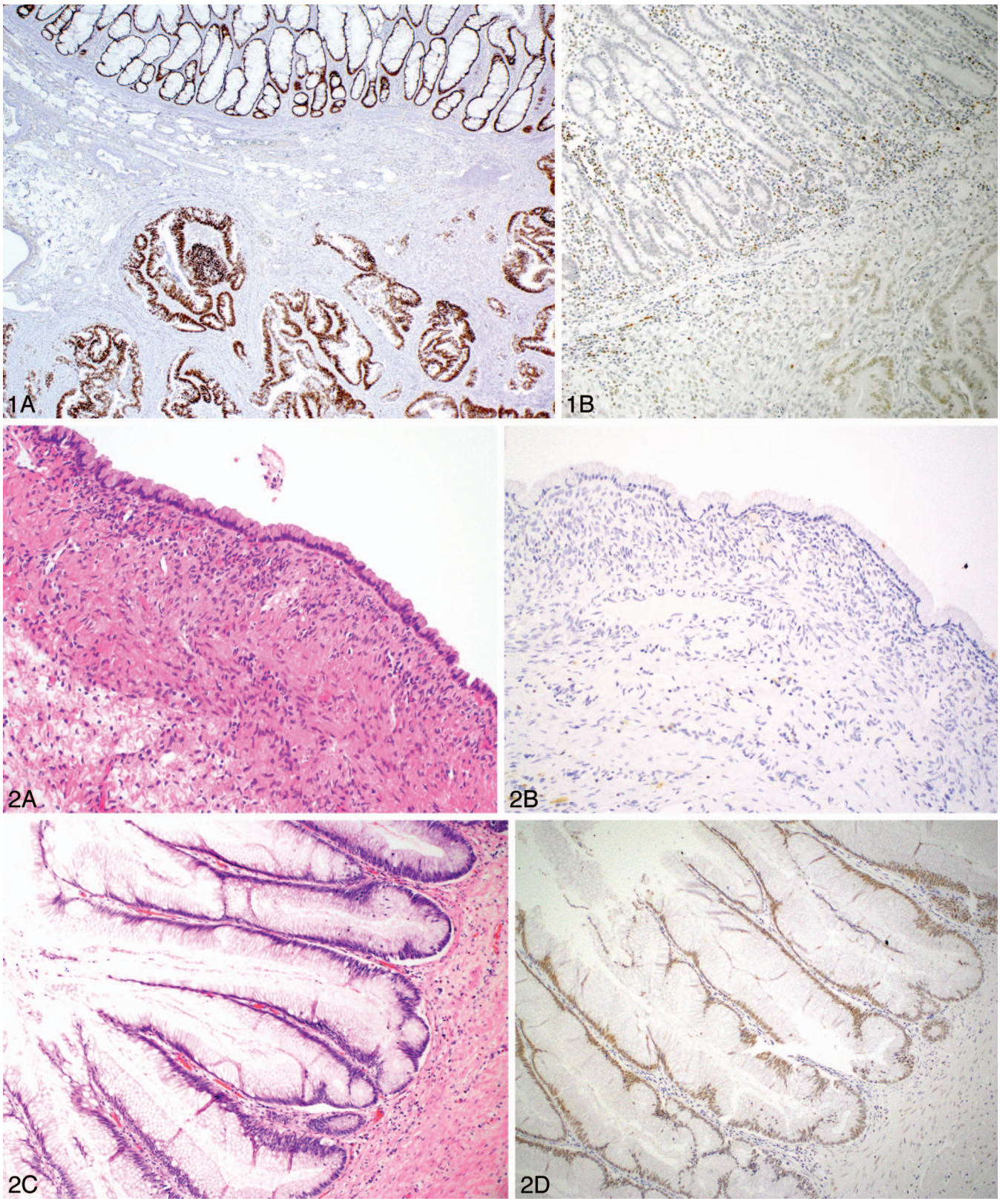

Figure 1. *Strong and diffuse SATB2 expression in colorectal adenocarcinoma (A), but patchy and weak expression in small intestinal adenocarcinoma (B). Note positive staining in nonneoplastic colonic mucosa (A) but negative staining in nonneoplastic small intestinal mucosa (B; original magnifications ×40 [A] and ×100 [B]).*

Figure 2. *Mucinous cystadenoma of the ovary (A) shows negative SATB2 staining (B), but low-grade appendiceal mucinous neoplasm/adenoma (C) shows diffuse SATB2 positivity (D; hematoxylin-eosin, original magnification ×200 [A and C]; original magnification ×200 [B and D]).*

Table 2. Scoring Criteria Used in Trastuzumab for Gastric Cancer (ToGA) Trial for Assessment of HER2 Expression in Gastric and Gastroesophageal Junction Adenocarcinomas by Immunohistochemistry

Score	Staining Pattern in Biopsy Specimen	Staining Pattern in Resection Specimen	Interpretation for HER2 Expression
0	No reactivity or no membranous reactivity in any tumor cell	No reactivity or membranous reactivity in <10% of tumor cells	Negative
1+	Tumor cell cluster[a] with a faint or barely perceptible membranous reactivity irrespective of percentage of tumor cells positive	Faint or barely perceptible membranous reactivity in ≥10% of tumor cells; cells are reactive only in part of their membrane	Negative
2+	Tumor cell cluster[a] with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells positive	Weak to moderate complete, basolateral, or lateral membranous reactivity in >10% of tumor cells	Equivocal[b]
3+	Tumor cell cluster[a] with a strong complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells positive	Strong complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells	Positive

[a] Tumor cell cluster consisting of ≥5 neoplastic cells.
[b] Fluorescent in situ hybridization analysis for *HER2* gene amplification should be performed.

of 17 mucinous adenocarcinomas, 0 of 3 endometrioid borderline tumors, and 0 of 72 endometrioid adenocarcinomas. Ovarian mucinous neoplasms also show a predominant $CK7^+/CK20^+$ pattern followed by a $CK7^+/CK20^-$ pattern. In contrast, low-grade appendiceal mucinous neoplasms frequently show a $CK7^-/CK20^+$ pattern followed by a $CK7^+/CK20^+$ pattern.[13] PAX8 is positive in 70% of ovarian tumors but negative in appendiceal neoplasms.[66]

Interpretation of HER2 Immunostain for Gastric and Gastroesophageal Junction Adenocarcinomas

Human epidermal growth receptor 2 (HER2, also referred to as ERBB2) is amplified and/or overexpressed in 9% to 27% of gastric and gastroesophageal junction adenocarcinomas.[67] This occurs more often in tumors with intestinal-type than diffuse-type morphology, and more often in well to moderately differentiated than poorly differentiated tumors. The Trastuzumab for Gastric Cancer (ToGA) trial demonstrated an increase in median overall survival in patients with advanced or metastatic gastric/gastroesophageal junction adenocarcinomas when treated with a humanized anti-HER2 monoclonal antibody trastuzumab plus chemotherapy compared with chemotherapy alone.[68] Thus, testing gastric and gastroesophageal junction adenocarcinomas for HER2 by immunohistochemistry on biopsy or resection specimens has become a routine practice for pathologists.[69] The HER2 immunohistochemistry scoring criteria as defined by the ToGA trial are summarized in Table 2.

HER2 expression in gastric and gastroesophageal junction adenocarcinomas can be complete, basolateral, or lateral membranous staining,[70] which differs from HER2 expression in breast carcinoma cells, where only complete membranous staining counts. Biopsies are scored differently from resection specimens because of tumor heterogeneity, with biopsy specimens requiring assessment of a tumor cell cluster (consisting of at least 5 tumor cells) regardless of the overall percentage of tumor cells involved. In contrast, a positivity cutoff of at least 10% of tumor cells is used for resection specimens. In general, strong (3+) positivity is visible to the naked eye or at low magnification (×2/×4). Weak to moderate (2+) positivity is visible at medium magnification (starting at ×10). Faint or barely perceptible (1+) positivity is only appreciated at high magnification (×40).[71] Examples of HER2 expression by immunohistochemistry are shown in Figure 3.

Care must be taken to assess only areas of adenocarcinoma, because epithelial cells with intestinal metaplasia, dysplasia, or reactive changes may also show membranous staining with HER2. Other potential sources of false-positive staining may include cytoplasmic staining with or without nuclear staining, and nonspecific pericellular and granular staining (particularly at tissue edges) instead of distinct intercellular membranous staining.[71]

Current guidelines recommend treatment with trastuzumab for tumors with an HER2 score of 3+, or a score of 2+ with evidence of *HER2* amplification by in situ hybridization (ISH). Trastuzumab is not recommended for tumors with a score of 0 or 1+.[72] Thus, fluorescent ISH testing (FISH) or other ISH methods should be performed for tumors that are 2+ by immunohistochemistry,[69] although both immunohistochemistry and FISH may be routinely performed on all cases in some institutions. When ISH is performed, it is important to evaluate the signals at the area that shows HER2 immunoreactivity.

Interpretation of Mismatch Repair Protein Immunostains

Because the clinical criteria used to identify patients with Lynch syndrome suffer from low sensitivity, recent guidelines recommend universal testing of all newly diagnosed CRCs by either microsatellite instability (MSI) analysis or immunohistochemistry for the expression of DNA mismatch repair (MMR) proteins MLH1, MSH2, MSH6, and PMS2.[73] Although the sensitivities and specificities of these 2 methods for Lynch syndrome are similar,[73] the advantages of immunohistochemistry include that it is more readily available, can be easily performed on small biopsy specimens, has faster turnaround time, and allows gene-specific sequencing analysis based on the staining pattern. The other clinical utility of MSI or MMR testing is to help guide patient management because MSI tumors tend to resist treatment with 5-fluorouracil but have a better stage-adjusted prognosis when compared with microsatellite-stable tumors.[74]

Functionally, MMR proteins act as heterodimers, with MLH1 pairing with PMS2, and MSH2 pairing with MSH6. MLH1 and MSH2 are obligate binding partners, such that abnormalities in either one of these proteins will result in loss of its respective secondary partner. As a result, loss of MLH1 protein is almost always accompanied by loss of PMS2; loss of MSH2 protein is almost always accompanied by loss of MSH6. In contrast, loss of PMS2 or MSH6 protein

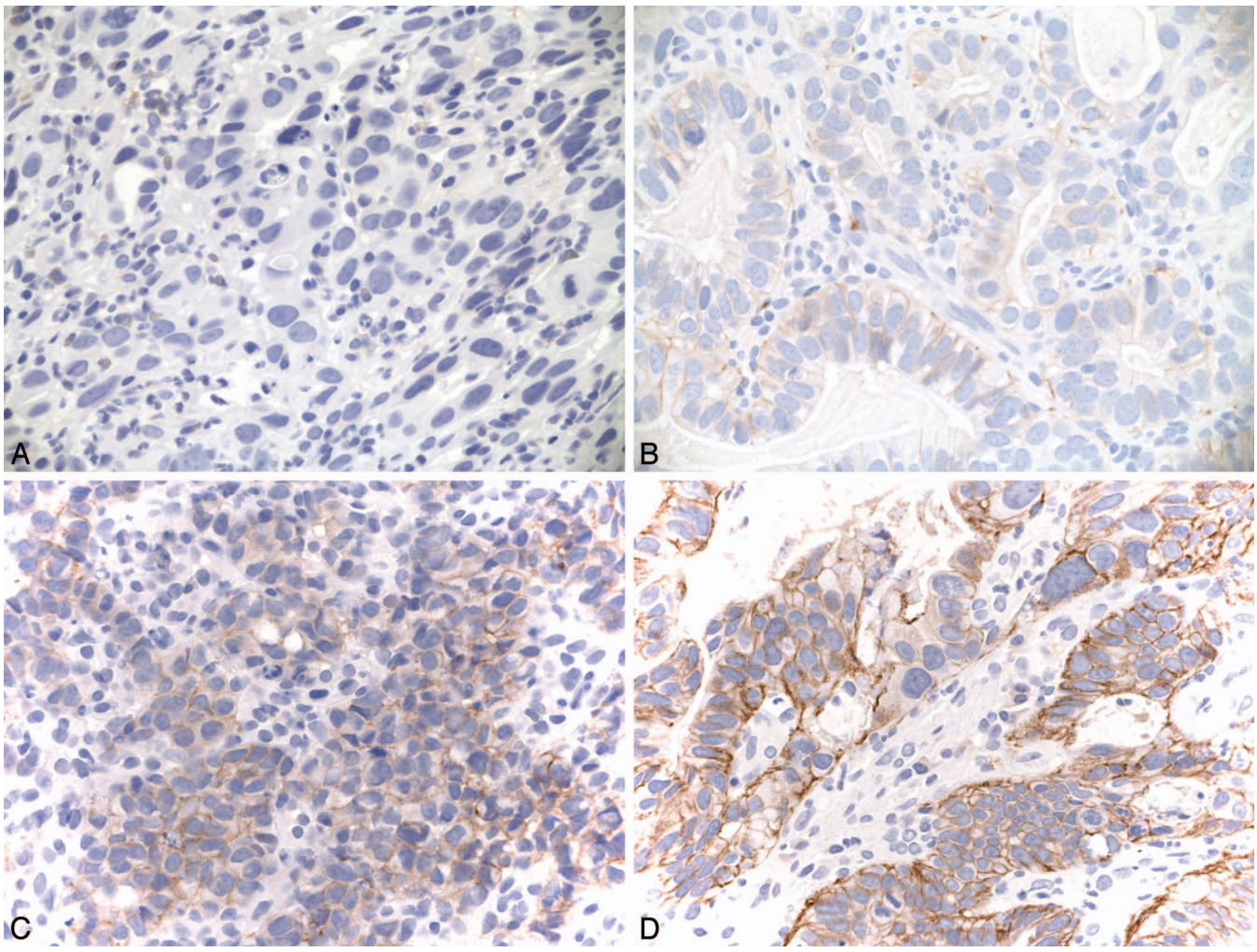

Figure 3. *Immunohistochemical assessment of HER2 expression on biopsy specimens: scores 0 (A), 1+ (B), 2+ (C), and 3+ (D; original magnification ×400).*

alone usually results from isolated mutations of the *PMS2* or *MSH6* gene, which does not affect MLH1 or MSH2 protein expression. In general, MMR immunostains are most useful in screening for mutations that result in protein truncation or degradation, which lead to absent expression of the protein. On the other hand, point mutations that result in nonfunctional but fully transcribed proteins with retained antigenicity may give rise to a false-normal staining pattern by immunohistochemistry.[75]

When MMR proteins are examined by immunohistochemistry, background inflammatory cells, stromal cells, and nonneoplastic epithelial cells should show positive nuclear staining, which serves as an internal control. Current understanding is that any positive staining in the nuclei of tumor cells should be considered intact (normal) expression. Patchy distribution of positive nuclear staining is not an uncommon finding in CRCs,[76] and thus patchy loss of nuclear expression in tumor cells is still considered intact expression. An interpretation of loss of expression should be made only if no nuclear staining is seen in all tumor cells and a positive reaction is present in internal control cells. It should be noted that immunohistochemical results can be affected by both biologic and technical factors. As mentioned above, staining variation within a tumor is common and may be attributable to tissue preservation affected by fixation time and/or the presence of regional ischemia/hypoxia in the tumor.[75] A few studies have also reported a nucleolar-only staining pattern, which should still be interpreted as loss of expression, or complete loss of MSH6 expression in tumors that have been treated with chemotherapy or radiation therapy.[77,78] In those situations, evaluation of pretreatment biopsy specimens should be helpful before proceeding to further genetic workup. Microsatellite instability analysis by polymerase chain reaction is another helpful option.

Loss of MLH1 expression can occur due to either germ line mutation of the *MLH1* gene, as seen for Lynch syndrome, or epigenetic inactivation of the *MLH1* gene promoter by hypermethylation, which results in MSI and is seen in approximately 12% of sporadic CRCs.[76] The V600E mutation of the *BRAF* gene is present in up to 70% of the sporadic MSI tumors with hypermethylation of the *MLH1* promoter but is not seen in Lynch-associated tumors. The presence of the V600E mutation is thus indicative of a sporadic MSI tumor and essentially excludes Lynch syndrome. Therefore, loss of MLH1 expression by immunohistochemistry should be followed by *BRAF* mutation analysis or *MLH1* promoter hypermethylation testing.

Table 3. Immunostaining Patterns of Mismatch Repair (MMR) Proteins, Interpretation, and Recommendations for Additional Workup

Staining Pattern					
MLH1	PMS2	MSH2	MSH6	Interpretation	Recommendation
+	+	+	+	1. Sporadic CRC 2. Non-Lynch hereditary CRC	1. None 2. MSI or other genetic testing if indicated by clinical and/or family history
−	−	+	+	1. Sporadic CRC with *MLH1* promoter hypermethylation 2. LS with *MLH1* germ line mutation 3. Rarely, LS with *PMS2* germ line mutation	1. *BRAF* mutation testing 2. *MLH1* methylation testing 3. Germ line testing for *MLH1* and/or *PMS2* if above test results are both negative 4. May consider somatic genetic testing if the above test results are all negative
+	+	−	−	1. LS with *MSH2* germ line mutation 2. LS with *EPCAM* germ line mutation[a] 3. Rarely, LS with *MSH6* germ line mutation 4. Sporadic CRC	1. Germ line testing for *MSH2* 2. Germ line testing for *EPCAM*, if the above test result is negative 3. Germ line testing for *MSH6*, if the above test results are negative 4. May consider somatic genetic testing if germ line test result is negative
+	−	+	+	1. LS with *PMS2* germ line mutation 2. Rarely, LS with *MLH1* germ line mutation	1. Germ line testing for *PMS2* 2. Germ line testing for *MLH1* if the above test result is negative 3. May consider somatic genetic testing if germ line test result is negative
+	+	+	−	1. LS with *MSH6* germ line mutation 2. Rarely, LS with *MSH2* germ line mutation 3. Sporadic CRC, treatment effects	1. Germ line testing for *MSH6* 2. Germ line testing for *MSH2* if the above test result is negative 3. May consider somatic genetic testing if germ line test result is negative
−	+	+	+	1. LS with *MLH1* germ line mutation 2. Rarely, LS with *PMS2* germ line mutation 3. Sporadic CRC	1. Germ line testing for *MLH1* 2. *BRAF* mutation or *MLH1* methylation if the above test result is negative 3. May consider somatic genetic testing if the above test results are all negative
+	+	−	+	1. LS with *MSH2* germ line mutation 2. LS with *EPCAM* germ line mutation 3. Sporadic CRC	1. Germ line testing for *MSH2* 2. Germ line testing for *EPCAM*, if the above test result is negative 3. May consider somatic genetic testing if germ line test result is negative
−	−	−	−	1. Germ line mutation in any *MMR* gene 2. *MSH2* germ line mutation with concurrent *MLH1* promoter hypermethylation 3. Sporadic CRC	1. Germ line testing for all 4 *MMR* genes 2. *BRAF* mutation or *MLH1* methylation if germ line test result for *MLH1* gene is negative 3. May consider somatic genetic testing if germ line test result is negative

Abbreviations: CRC, colorectal cancer; LS, Lynch syndrome; MSI, microsatellite instability; +, intact nuclear expression; −, loss of nuclear expression.

[a] *EPCAM* (epithelial cell adhesion molecule) is a gene located just upstream from the *MSH2* gene. Deletions of the terminal codon of the *EPCAM* gene result in silencing of the *MSH2* gene, leading to a phenotype very similar to LS.

Vastly diminished or heterogeneous staining with MSH6 has been seen in association with loss of MLH1 expression (secondary to either *MLH1* germ line mutation or promoter hypermethylation). This is usually caused by somatic mutations of the *MSH6* gene and is not associated with germ line *MSH6* mutation.[79,80] A case of "null pattern" has also been reported, where loss of expression is observed in all 4 MMR proteins due to germ line *MSH2* mutation and concurrent *MLH1* promoter hypermethylation.[81] Furthermore, rare cases of *MLH1* promoter hypermethylation with simultaneous *MLH1* or *MSH2* germ line mutation have been described.[82] These rare phenomena should be considered if unusual staining patterns are encountered, and the addition of MSI testing may be helpful to confirm the immunohistochemical findings. Interpretations of various MMR protein immunostaining patterns and recommendations for further workup are summarized in Table 3.

BRAF Analysis

As mentioned earlier, *BRAF* mutation analysis is a useful test to separate sporadic MSI tumors from Lynch-associated tumors. The test also has therapeutic implications because the presence of mutation is associated with limited response to EGFR-targeted therapies in CRC patients. Recently, a V600E mutant-specific antibody (clone VE1) has become available, which shows cytoplasmic staining in tumor cells harboring this specific mutation. However, a number of studies have shown that the antibody suffers from suboptimal sensitivity and specificity, with significant discordances with molecular results in CRCs.[83–85] Standardization of multiple technical factors, such as specimen fixation time, antigen retrieval methods, antibody optimization, and scoring criteria, appears necessary to make this antibody useful in clinical practice.[83,86,87] Overall, the currently available data suggest that the VE1 antibody

should not be used as a substitute for molecular testing for the detection of *BRAF* V600E mutation in CRC specimens.

Examples

An 87-year-old woman presented with a 12-cm mass in the right colon, which adhered to the right ovary and abdominal sidewall as shown by imaging studies. Serum CEA and CA125 were both elevated. Colonoscopy showed a partially obstructing mass in the proximal ascending colon with ulceration. Biopsy of the mass showed sheets of neoplastic cells involving the mucosa and submucosa. The neoplastic cells exhibited large round or oval nuclei, vesicular chromatin, prominent nucleoli, moderate amounts of amphophilic cytoplasm, indistinct cell borders, and frequent mitoses (Figure 4, A). Occasional intratumoral lymphocytes were noted. Histologic differentials included large cell neuroendocrine carcinoma, medullary carcinoma of colonic primary, metastatic undifferentiated carcinoma/sarcoma, lymphoma, and metastatic melanoma. Initial immunohistochemical workup demonstrated the tumor cells to be diffusely positive for AE1/AE3; patchy positive for calretinin; and negative for CK7, CK20, CDX2, villin, CD45, S100, PAX8, and synaptophysin. Additional immunostains showed diffuse positivity for SATB2 (Figure 4, B) and CDH17 (Figure 4, C), and loss of MLH1 and PMS2 immunoreactivity. The resection specimen showed poorly differentiated carcinoma with pushing borders and peritumoral inflammatory response (Figure 4, D), consistent with medullary carcinoma of the colon. In this case, SATB2 and CDH17 were very helpful in confirming the colonic origin of the tumor. Further workup showed the presence of *BRAF* V600E mutation in tumor cells, indicating a sporadic nature.

IMMUNOMARKERS FOR HEPATOCELLULAR NEOPLASMS

Pathologists often face the challenge of distinguishing well-differentiated hepatocellular carcinoma (HCC) from benign hepatocellular masses or nodular lesions, such as hepatocellular adenoma, and poorly differentiated HCC from nonhepatocellular neoplasms, such as metastatic adenocarcinoma. Immunomarkers thus play an indispensable role when difficult cases are encountered (Table 4).[88–90]

Hepatocellular Markers

CEA and CD10.—Polyclonal anti-CEA antibodies (pCEAs) cross-react with a biliary glycoprotein, giving rise to a characteristic canalicular staining pattern in normal liver. This canalicular pattern is preserved in most HCC cases (Figure 5), with a sensitivity of 70% to more than 90% in some studies.[91,92] In contrast, adenocarcinomas, including cholangiocarcinoma, typically exhibit a diffuse cytoplasmic staining pattern. However, poorly differentiated HCC may also show cytoplasmic positivity and lack canalicular immunoreactivity, which significantly limits its use in the distinction between poorly differentiated HCC and adenocarcinoma. Using monoclonal anti-CEA antibodies (mCEAs) may help in this regard because mCEAs may be positive in adenocarcinoma but are less likely to be positive in HCC.[93,94] Caution should also be exercised not to interpret membranous staining as the canalicular pattern, which can be seen in adenocarcinomas.

Similar to pCEA, CD10 also shows a canalicular pattern in neoplastic and nonneoplastic liver tissues, but it appears to be less sensitive for HCC in comparison with pCEA.[91,92,95–97] Unlike pCEA, however, CD10 appears less likely to show cytoplasmic positivity, which gives a cleaner background for easier interpretation.

Hepatocyte Antigen.—Hepatocyte antigen (Hep Par 1) stains both neoplastic and nonneoplastic liver tissues, with a sensitivity of more than 70% for HCC.[91,92,97–100] Hep Par 1–negative HCCs tend to be poorly differentiated. Some positive cases may show patchy staining, which reduces its diagnostic value on biopsy specimens and fine-needle aspirations. Although uncommon, a small fraction of adenocarcinomas can also express Hep Par 1, which may create diagnostic dilemmas when poorly differentiated intrahepatic cholangiocarcinomas or metastatic adenocarcinomas are encountered.[91,97,101] Of note, Hep Par 1 antigen is a urea cycle enzyme carbamoyl phosphate synthetase 1 in the mitochondria.[102]

Arginase-1.—Arginase-1 (Arg-1) is also a urea cycle enzyme expressed in neoplastic and nonneoplastic liver tissues. In the initial study, Yan et al[103] reported a sensitivity of 96% for HCC. Even for poorly differentiated HCC, the sensitivity was as high as 86%. Only rare cases of adenocarcinoma were found to be positive for this marker. Indeed, subsequent studies have confirmed that Arg-1 is currently the best available hepatocellular marker, with a sensitivity around 90%.[104–106] In comparison with Hep Par 1, Arg-1 is much less frequently positive in adenocarcinomas.[103–107] Like Hep Par 1, positive Arg-1 staining can also be patchy.

Albumin RNA ISH.—No diagnostic immunomarkers for albumin are currently available. However, recent investigations have shown that branched-chain RNA ISH for albumin is a promising diagnostic tool for HCC.[108] According to the authors, the sensitivity for HCC (including poorly differentiated HCC) is 99%. The only non-HCC carcinoma that is also positive for albumin with a similarly high frequency is intrahepatic cholangiocarcinoma,[109] but this should be easily distinguished from HCC if Arg-1 is also included in the workup panel.[108] Otherwise, only rare cases of pancreatic acinar cell carcinoma (including mixed acinar ductal carcinoma and mixed acinar neuroendocrine carcinoma) have been found to show albumin positivity.[110] The test can thus be very useful in the distinction between intrahepatic carcinomas and metastatic carcinomas to the liver. Of note, nonneoplastic hepatocytes and bile ducts are also positive for albumin RNA.[111]

Malignant Hepatocellular Markers

Glypican-3.—Glypican-3 (GPC3) is an oncofetal protein that can be detected in 70% to 80% of HCCs but not in benign hepatocellular lesions, such as hepatocellular adenoma.[112–114] Positive stains can be cytoplasmic, membranous, canalicular, and/or cytoplasmic dotlike. Immunoreactivity is often heterogeneous within the tumor and is frequently focal/patchy and weak in well-differentiated HCC. It has been shown that GPC3 sensitivity can be as high as 80% to 90% for moderately and poorly differentiated HCC, but as low as 50% to 67% for well-differentiated HCC. It is thus conceivable that these staining characteristics have a considerable effect when GPC3 is used to distinguish a well-differentiated HCC from a benign hepatocellular lesion on a core biopsy. In fact, the detection rate is usually below 50% when studies are performed on biopsy or fine-needle aspiration specimens.[105,115] Another pitfall is that focal GPC3 expression can be detected in a small fraction of cirrhotic nodules,[112,116] and thus the diagnosis of HCC should not be based on GPC3 positivity

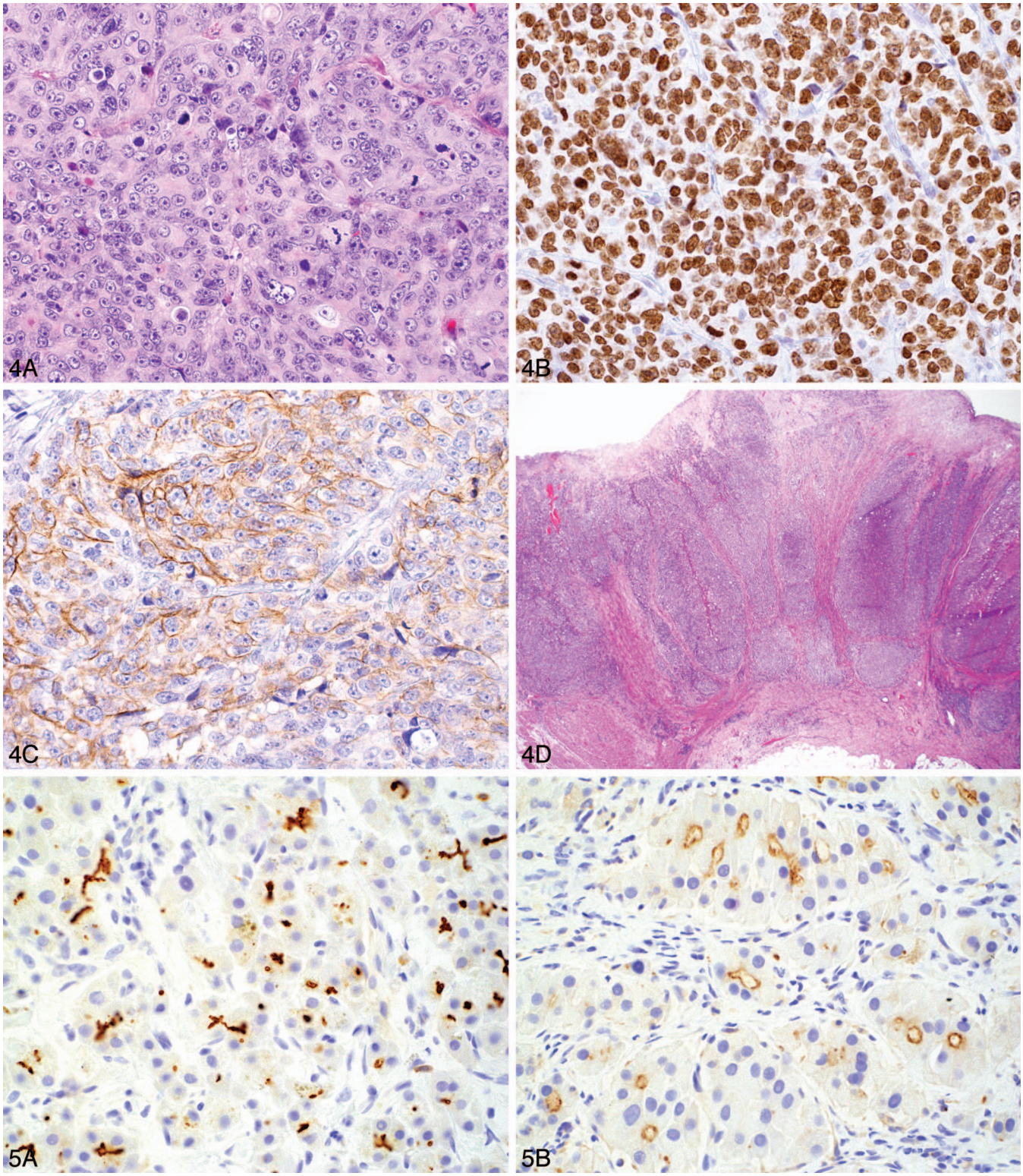

Figure 4. *A biopsy of a right colon mass shows poorly differentiated carcinoma on histologic examination (A). By immunohistochemistry, tumor cells are positive for SATB2 (B) and CDH17 (C). The subsequent resection specimen shows features of medullary carcinoma of the colon (D; hematoxylin-eosin, original magnifications ×400 [A] and ×20 [D]; original magnification ×400 [B and C]).*

Figure 5. *A case of moderately differentiated hepatocellular carcinoma showing characteristic canalicular staining pattern for polyclonal carcinoembryonic antigen (pCEA; A), which may show a luminal pattern when there is pseudoacinar formation (B; original magnification ×400).*

Table 4. Diagnostic Markers for Hepatocellular Tumors	
	Staining Pattern
Hepatocellular markers	
Polyclonal CEA	Canalicular
CD10	Canalicular
Hepatocyte antigen	Cytoplasmic
Arginase-1	Cytoplasmic, with or without nuclear staining
Albumin (in situ hybridization)	Cytoplasmic
Malignant hepatocellular markers	
Glypican-3	Cytoplasmic
Glutamine synthetase	Cytoplasmic
Heat shock protein 70	Cytoplasmic and nuclear
CD34	Sinusoidal
α-Fetoprotein	Cytoplasmic
Clusterin	Canalicular (enhanced)

Abbreviation: CEA, carcinoembryonic antigen.

alone. Finally, GPC3 expression is not HCC specific. Frequent expression has been documented in a number of neoplasms, such as hepatoblastoma,[117] undifferentiated embryonal sarcoma of the liver,[118] yolk sac tumor,[119,120] choriocarcinoma,[121] pancreatic acinar cell carcinoma,[110,121] Merkel cell carcinoma,[122] and lung squamous cell carcinoma.[123] Positive staining has also been reported in a small fraction of adenocarcinomas of the esophagus, stomach, small bowel, and colon.[121] GPC3 expression is seen in only rare cases of cholangiocarcinoma, which is typically focal and weak. In fact, all published studies have shown negative GPC3 expression in cholangiocarcinomas except for one that showed positive staining in up to 13% of cases.[124] Pancreatic ductal adenocarcinomas are also negative, but only a limited number of cases have been examined.

Glutamine Synthetase.—In normal liver, glutamine synthetase (GS) expression is restricted to centrilobular (zone 3) hepatocytes,[125] positive in only one to a few hepatic plates around central veins (Figure 6, A, inset). This characteristic pattern is not maintained in cirrhotic livers. Recent studies have demonstrated that GS is a useful immunomarker for both benign and malignant hepatocellular neoplasms. Strong GS expression in the cytoplasm of hepatocytes, with positive cells forming large sheets arranged in an anastomosed geographic or "maplike" pattern, is essentially diagnostic of focal nodular hyperplasia (FNH).[126,127] Glutamine synthetase is usually not expressed in hepatocytes around fibrous scars containing arteries and bile ductules. This unique "maplike" pattern is seen in virtually all FNH cases, including those that are morphologically uncharacteristic (Figure 6, A). Hepatocellular adenomas are either negative or positive around veins, or show patchy staining with no distinctive patterns. Patchy staining may sometimes be confused with the "maplike" pattern, but positively stained hepatocytes are usually present at the periphery of adenomas. In addition, the staining may not be as strong or uniform as that seen for FNH. An exception is β-catenin–activated adenomas, which usually show strong and diffuse (defined as >50% of tumor cells positively stained) GS expression. This may or may not be accompanied by nuclear β-catenin staining. Glutamine synthetase is positive in 70% of HCC cases, but only approximately 50% of positive cases show a strong and diffuse staining pattern (Figure 6, B).[128] Despite the general belief that upregulation of GS expression is the result of β-catenin nuclear translocation, there is a lack of direct correlation between strong and diffuse GS immunostaining and β-catenin exon 3 mutations in more than 50% of hepatocellular adenomas and HCCs.[129] Glutamine synthetase does not appear to be a good marker to distinguish hepatocellular from nonhepatocellular neoplasms because its expression has been demonstrated in 76% of intrahepatic cholangiocarcinomas and 71% of hepatic metastatic tumors,[130] as well as 100% of solid pseudopapillary neoplasms of the pancreas.[131]

Heat Shock Protein 70.—Heat shock protein 70 (HSP70) was the most abundantly upregulated gene in early HCC in one of the gene expression profiling studies.[132] Nuclear and cytoplasmic HSP70 immunoreactivity has been reported in 70% of HCCs (Figure 7, A); it is usually patchy, with an intermediate staining intensity. Diffuse positivity is seen in only one-third of cases.[128] As expected, the frequency of positivity is less than 50% on biopsy specimens.[133] HSP70 expression in HCC does not appear to correlate with tumor differentiation, which is a useful feature when the differential diagnosis involves well or poorly differentiated HCC. It is normally expressed in biliary epithelium, which can be used as an internal control. In our experience, HSP70 is a less ideal marker, not only because of its low sensitivity on biopsies, but also for the difficulty of interpretation, because benign hepatocytes, particularly those in cirrhotic nodules, can also be positively stained in some cases (Figure 7, B). Stronger staining is thus required when compared with nonneoplastic hepatocytes if they are also present in the same biopsy. Similar to GS, HSP70 is frequently expressed in cholangiocarcinomas (88%) and metastatic tumors, such as CRCs,[130] which limits its utility in the distinction between hepatocellular and nonhepatocellular neoplasms.

CD34.—CD34 is diagnostically useful because it shows different staining patterns in neoplastic and nonneoplastic liver tissues. In normal and cirrhotic livers, it stains endothelial cells lining blood vessels in the portal tracts and fibrous septa. Sinusoidal spaces are largely negative, except for those immediately adjacent to portal tracts or fibrous septa that may show positive staining (Figure 8, A). In contrast, a diffuse sinusoidal staining pattern is characteristic of HCC (Figure 8, B), seen in 95% of cases.[114,134] Hepatocellular adenomas predominantly show patchy sinusoidal staining (Figure 8, C), but diffuse staining can be seen in approximately 20% of cases. Patchy staining with periseptal accentuation is also common for FNH.[89,114]

α-Fetoprotein.—Although serum α-fetoprotein (AFP) levels are frequently elevated in patients with HCC, its detection rate on tissue sections is only around 30%.[91,97] It is frequently positive in hepatoblastomas and yolk sac tumors. Expression of AFP is uncommon in cholangiocarcinomas and metastatic carcinomas to the liver, but this has been well documented.[97,135]

Clusterin.—Clusterin is a multifunctional glycoprotein implicated in numerous biologic processes, such as programmed cell death, lipid transport, cell adhesion, membrane recycling, complement regulation, senescence, tumorigenesis, and cancer chemoresistance.[136] A few studies have demonstrated a canalicular clusterin staining pattern in 54% to 75% of HCCs.[137–139] Strong clusterin expression is also seen on the luminal surface of pseudoacini in HCC cases.[139,140] This canalicular pattern appears unique to HCC because it is much enhanced (Figure 9, A) in comparison with benign hepatocellular nodular lesions, such as hepa-

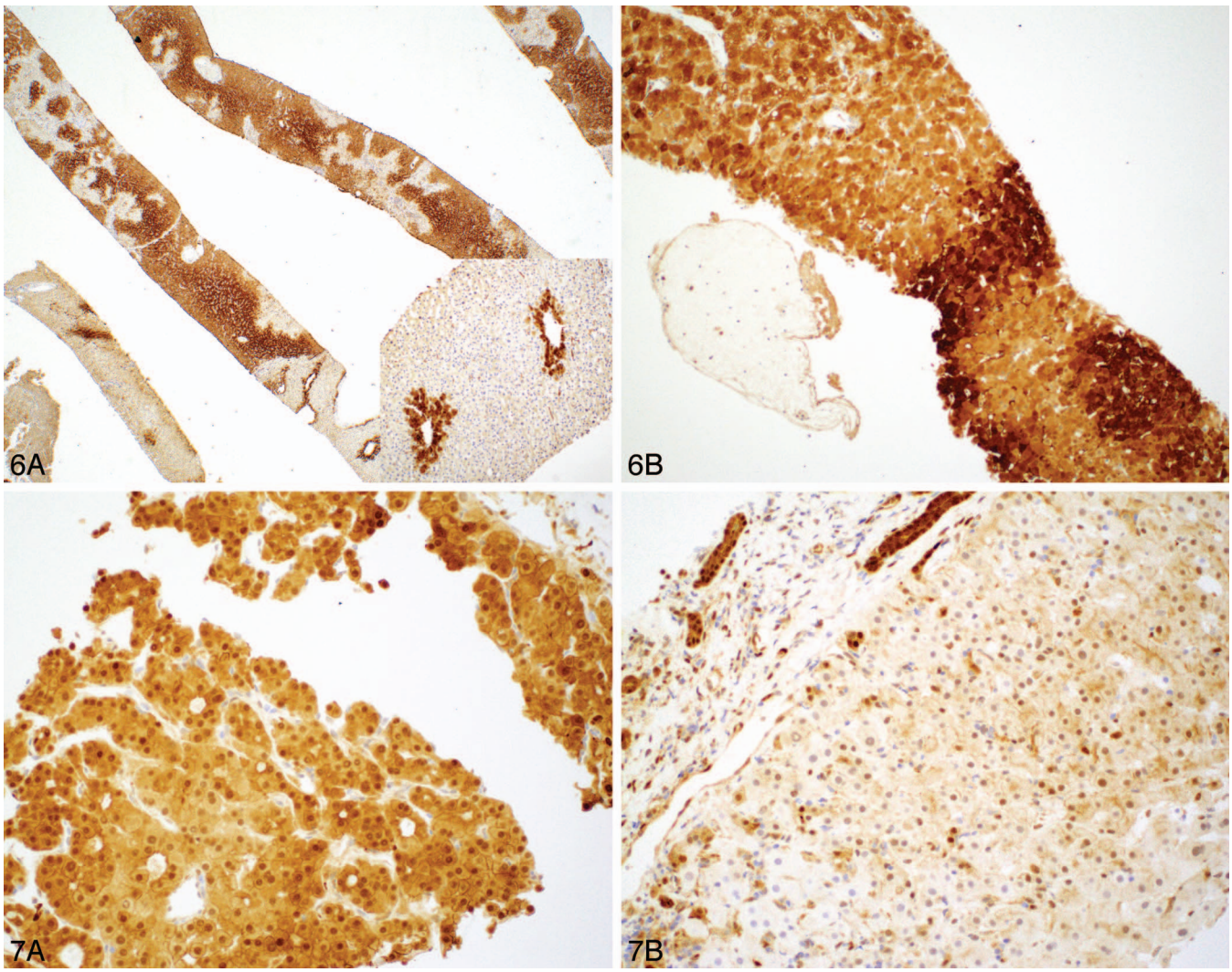

Figure 6. *Immunostaining for glutamine synthetase shows a characteristic "maplike" pattern for focal nodular hyperplasia on a liver core biopsy (A). Normal liver shows immunoreactivity in only one to a few layers of hepatocytes around central veins (inset). Diffuse strong cytoplasmic staining is typically seen in hepatocellular carcinoma (B), as shown in this case, and a subset of hepatocellular adenoma (original magnifications ×20 [A] and ×200 [A inset, and B]).*

Figure 7. *Positive nuclear and cytoplasmic HSP70 immunostaining seen in a case of well-differentiated hepatocellular carcinoma (A). Positive staining is also observed in hepatocytes in this cirrhotic nodule (B), which may be weaker in comparison with hepatocellular carcinoma if both components are present in the same biopsy. Note stronger immunoreactivity in benign bile ducts and ductules (original magnification ×400).*

tocellular adenoma, FNH, and large regenerative nodules, and has not been observed in nonhepatocellular neoplasms examined thus far.[141] Cytoplasmic immunoreactivity is seen in both malignant and benign hepatocytes. Benign hepatocytes may also show canalicular staining, but it is always delicate, granular, and with a "railroad track"–like pattern (Figure 9, B). Our preliminary data have shown that clusterin is superior to pCEA and CD10 in that it may help distinguish not only hepatocellular from nonhepatocellular origins, but also malignant from benign hepatocellular nodular lesions (H.L.W., unpublished data, 2016). The enhanced canalicular pattern is less frequently observed in poorly differentiated HCCs in comparison with well-differentiated or moderately differentiated ones.

Immunomarkers for Fibrolamellar HCC

In comparison with conventional HCC, fibrolamellar variant is usually negative for AFP, less frequently positive for GPC3, and more frequently positive for CK7.[142,143] One study also showed CD68 expression in 31 of 33 fibrolamellar samples (97%) from 24 patients, in contrast to 10 of 39 conventional HCCs (27%) arising in noncirrhotic livers and 3 of 27 HCCs (11%) arising in cirrhotic livers.[144] The staining is cytoplasmic, typically with a granular or stippled pattern. This marker may be useful to help separate fibrolamellar from scirrhous HCCs that may cause diagnostic confusion on core biopsies.[145]

Immunophenotypic Classification of Hepatocellular Adenoma

Advances in molecular studies have subdivided hepatocellular adenoma into 4 different types: HNF1α inactivated, β-catenin activated, inflammatory, and unclassified. This topic has been reviewed extensively in previous publications.[146–149] Briefly, HNF1α-inactivated adenoma is characterized by diffuse steatosis histologically, somatic or germ

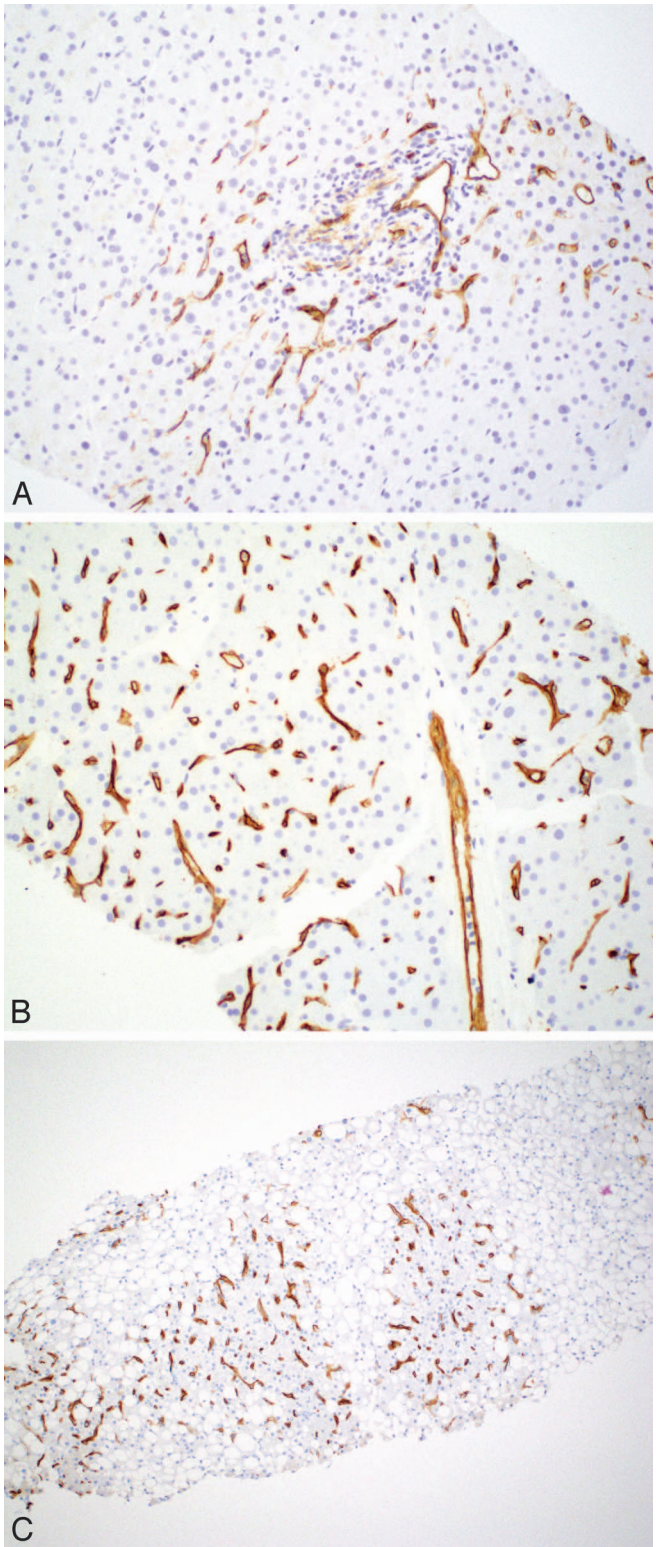

Figure 8. *CD34 highlights portal vasculature and sinusoids immediately adjacent to portal tract in nonneoplastic liver (A), but it shows a diffuse (also termed "complete") sinusoidal staining pattern (B) in hepatocellular carcinoma because of capillarization associated with hepatotumorigenesis, or patchy (also termed "incomplete") sinusoidal pattern (C) seen in most hepatocellular adenomas (original magnifications ×400 [A and B] and ×200 [C]).*

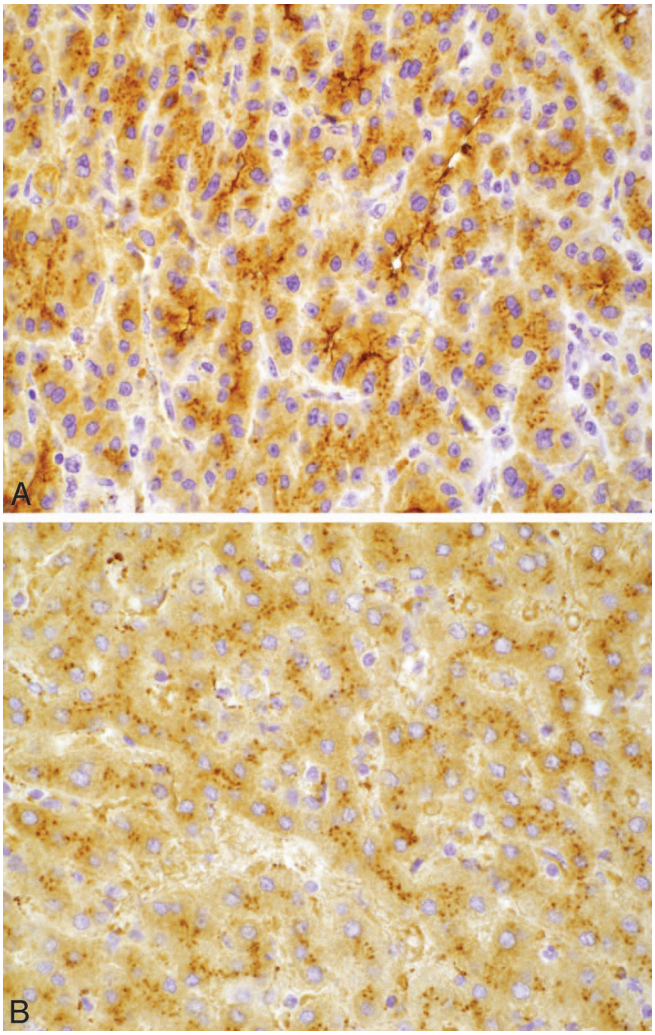

Figure 9. *Clusterin shows an enhanced canalicular staining pattern in a hepatocellular carcinoma (A) but a delicate, granular canalicular pattern in adjacent nonneoplastic (cirrhotic) liver (B). Note that cytoplasmic staining is seen in both malignant and benign hepatocytes (original magnification ×400).*

line mutations of the *HNF1α* gene encoding hepatocyte nuclear factor-1α, and loss of expression of liver–fatty acid binding protein by immunohistochemistry. β-Catenin–activated variant frequently shows nuclear atypia and harbors mutations of the *CTNNB1* gene. Immunophenotypically, this variant shows strong and diffuse GS expression, with or without nuclear β-catenin staining. Identification of this variant is important because of its strong association with the development of HCC. Inflammatory adenoma was previously termed "telangiectatic FNH." It is characterized by focal or diffuse inflammatory cell infiltration, sinusoidal dilatation, and ductular reaction. Approximately 80% of inflammatory adenomas have mutations of the *IL6ST*, *JAK1*, *STAT3*, *FRK*, or *GNAS* genes. Up to 10% of cases also have concomitant *CTNNB1* mutations. The diagnostic immunomarkers for this variant are serum amyloid A and/or C-reactive protein, which typically show diffuse cytoplasmic immunoreactivity. The adjacent nonneoplastic hepatocytes may also show positive staining for these 2 markers, but the staining is usually patchy and less intense.

Table 5. Useful Immunomarkers for Distinguishing Well-Differentiated Hepatocellular Carcinoma (WDHCC) From Hepatocellular Adenoma (HCA) and High-Grade Dysplastic Nodule (HGDN)

Marker	WDHCC, %	HCA, %	HGDN, %
Glypican-3	50–69	0	<10
Glutamine synthetase (strong and diffuse)	35–60	10-15	<15
Heat shock protein 70	40–78	0	<10
Positive in at least 2 of the above 3 markers	50–72	0	0
CD34 (diffuse sinusoidal pattern)	95[a]	20	+/−
Clusterin (enhanced canalicular pattern)	75	0	No data
CK7	Absence of ductules at border (invasive growth)	No value	Presence of ductules at border (noninvasive growth)

Abbreviations: CK, cytokeratin; +/–, may or may not.

[a] This number is based on all hepatocellular carcinomas. The % appears lower for early hepatocellular carcinoma.

Distinction Between Inflammatory Hepatocellular Adenoma and FNH

As mentioned above, ductular reaction may be present in inflammatory adenoma, which can be confused with FNH on core biopsies. Immunomarkers that are most useful for the distinction are GS and serum amyloid A. As shown in Figure 6, A, GS shows a characteristic "maplike" pattern for FNH. In contrast, adenomas are either negative or positive around veins, or show patchy staining with no distinctive patterns. Strong and diffuse GS staining with or without nuclear β-catenin localization may be seen in inflammatory adenomas with concomitant *CTNNB1* mutations. Serum amyloid A is positive in adenomas but usually negative in FNH. C-reactive protein appears to be less specific according to 1 study, which demonstrated positivity in 78% of FNHs, with diffuse staining in 15% of cases.[126] This study also showed positive serum amyloid A staining in 7 of 40 FNHs, 5 of which were diffusely stained. The "maplike" GS pattern is thus most useful for the distinction.[127,150]

Distinction Between Well-Differentiated HCC and Hepatocellular Adenoma

Histologic distinction between HCC and hepatocellular adenoma is a well-known diagnostic challenge.[138,146] This is particularly true when HCC is well differentiated and when diagnostic material is limited, as in a core biopsy. In addition to reticulin stain, a number of immunomarkers have been shown to be of value in aiding in the distinction (Table 5). Among these, GPC3 appears to have been better investigated; it has never been found to be positive in adenomas.[112–114,151] HSP70 has also been reported to be negative in adenomas in 2 small series.[151,152] Despite their low sensitivity to detect HCC on core biopsies, both GPC3 and HSP70 appear to be useful in the distinction between HCC and hepatocellular adenoma, given their high specificity. The unique staining patterns of CD34 and clusterin may also be useful. Interestingly, in our experience the enhanced canalicular clusterin pattern has never been observed in hepatocellular adenomas.[138,139] Other immunomarkers, such as proliferating cell nuclear antigen and insulin-like growth factor 2, are also potentially useful, but both neoplastic and nonneoplastic hepatocytes need to be present for their interpretation.[138,139] Glutamine synthetase does not appear to be a good marker for this distinction because the strong and diffuse staining patterns can be seen in both HCC and adenoma.

Distinction Between Early HCC and Dysplastic Nodule

Dysplastic nodules (DNs) are believed to be precursor lesions for HCC. These nodular lesions are typically detected in cirrhotic livers, usually are approximately 1 cm in size, and are distinctive from surrounding cirrhotic nodules grossly in terms of color and/or texture.[153] High-grade DNs (HGDNs) show at least moderate cytologic or architectural atypia, but the abnormalities are insufficient for the diagnosis of HCC. It is thus histologically challenging to differentiate HGDN from early HCC. One of the useful immunomarkers is CK7, which helps highlight the areas of stromal invasion in early HCC by the absence of ductular reaction at tumor borders. In contrast, ductules are present circumferentially around the HGDN because it lacks invasive growth.[154] CK19 is similarly useful in this regard.

Di Tommaso et al[128] compared 22 HGDNs with 32 early or well-differentiated HCCs, and found GPC3, GS, and HSP70 expression in 69%, 59% and 78% of HCCs, in contrast to 9%, 14% and 5% of HGDNs, respectively. The expression of these markers in HGDNs was focal (positive in <50% of cells). Interestingly, up to 72% of HCC cases showed positivity for at least 2 of the 3 markers. This was in marked contrast to HGDNs, which were never positive for 2 markers simultaneously.[128,133] The authors thus recommend using these 3 markers as a panel because it provides a very high specificity (100%) for HCC if a liver lesion is positive for 2 of the markers. These observations have been validated subsequently by similar studies.[152,155]

The value of CD34 immunostaining in the distinction between early HCC and HGDN has been investigated in a few early studies. It appears that the extent of sinusoidal capillarization in DNs varies between those in cirrhotic nodules and those in HCCs.[156–158] Therefore, DNs may exhibit a diffuse sinusoidal pattern, albeit less frequently than HCCs. On the other hand, early HCCs may show a patchy pattern because they are still at the early stage as a carcinoma.

Distinction Between Hepatoid Carcinoma and HCC

Hepatoid carcinoma is a unique type of extrahepatic adenocarcinoma with a significant component or entire tumor showing hepatocellular differentiation. It can originate from various organs, such as the stomach, pancreas, gallbladder, esophagus, and colon. In comparison with HCCs, hepatoid carcinomas are more frequently positive for AFP (92%) by immunohistochemistry (most of these patients have elevated serum AFP levels), more frequently positive for CK19 (100%), and less frequently positive for

Hep Par 1 (38%).[159] A high proportion of hepatoid carcinomas are also positive for pancytokeratin AE1/AE3 (92%) and GPC3 (100%). Canalicular pCEA and CD10 stains are seen in two-thirds of cases. Of note, only approximately 30% and approximately 10% of HCCs are positive for AE1/AE3 and CK19, respectively, and CK19 expression has been regarded as a predictive marker for poor prognosis in patients with HCC. Hepatoid carcinomas may also express MOC31 and mCEA, which are infrequently expressed in HCCs.[160]

In a recent study on 8 hepatoid carcinomas, Chandan et al[161] demonstrated positive stains for Hep Par 1, Arg-1, GPC3, and AFP in 8 (100%), 5 (62.5%), 4 (50%), and 4 (50%) cases, respectively. Canalicular pCEA pattern was observed in 3 cases (37.5%). Albumin ISH was performed on 4 cases, and 3 (75%) showed positivity. These additional findings indicate that hepatoid carcinoma is essentially indistinguishable from HCC not only histologically but also immunophenotypically. The distinction can only be made reliably based on their locations of origin.

Examples

A 56-year-old woman presented with diffuse lymphadenopathy throughout the neck, chest, abdomen, and pelvis, and innumerable sclerotic osseous metastases of uncertain primary. Ultrasound-guided biopsy of the right inguinal lymph node was performed. Sections showed neoplastic epithelial cells arranged in trabeculae or clusters, with frequent acinar structures. Occasional isolated arterioles were noted, but there was no significant intervening stroma present. Tumor cells had round nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm containing variably sized fat vacuoles (Figure 10, A and B). No bile production was appreciated. Although the histologic features highly resembled those of HCC, immunostains were negative for hepatocellular markers Hep Par 1, Arg-1, pCEA, and CD10. AFP was also negative. Tumor cells were positive for pancytokeratin, CK7, GATA3 (Figure 10, C), and GCDFP15, suggesting a breast primary. Further imaging workup showed in the left breast an ill-defined hypoechoic region with increased vascularity and architectural distortion. A core biopsy of the breast lesion confirmed the diagnosis of mammary carcinoma with apocrine features. No liver lesions were detected by imaging.

IMMUNOMARKERS FOR PANCREATICOBILIARY CARCINOMAS

A major challenge in diagnostic pancreaticobiliary pathology is to distinguish adenocarcinomas of the pancreas and bile duct from benign/reactive ductal epithelium on biopsy or cytology specimens. The diagnostic materials from these locations are typically limited because of the difficulty of endoscopic sampling and may show crush artifacts. Bile duct specimens also often show epithelial reactive changes, fibrosis, and compounding inflammation after stent placement, which make the histologic evaluation even more difficult. Several emerging immunomarkers are available, which can be of help.[3] Immunomarkers may also be used to help distinguish intrahepatic cholangiocarcinoma from hepatic metastasis of pancreatic ductal adenocarcinoma, distinguish pancreatic acinar cell carcinoma from other solid pancreatic neoplasms, and subclassify ampullary adenocarcinoma.

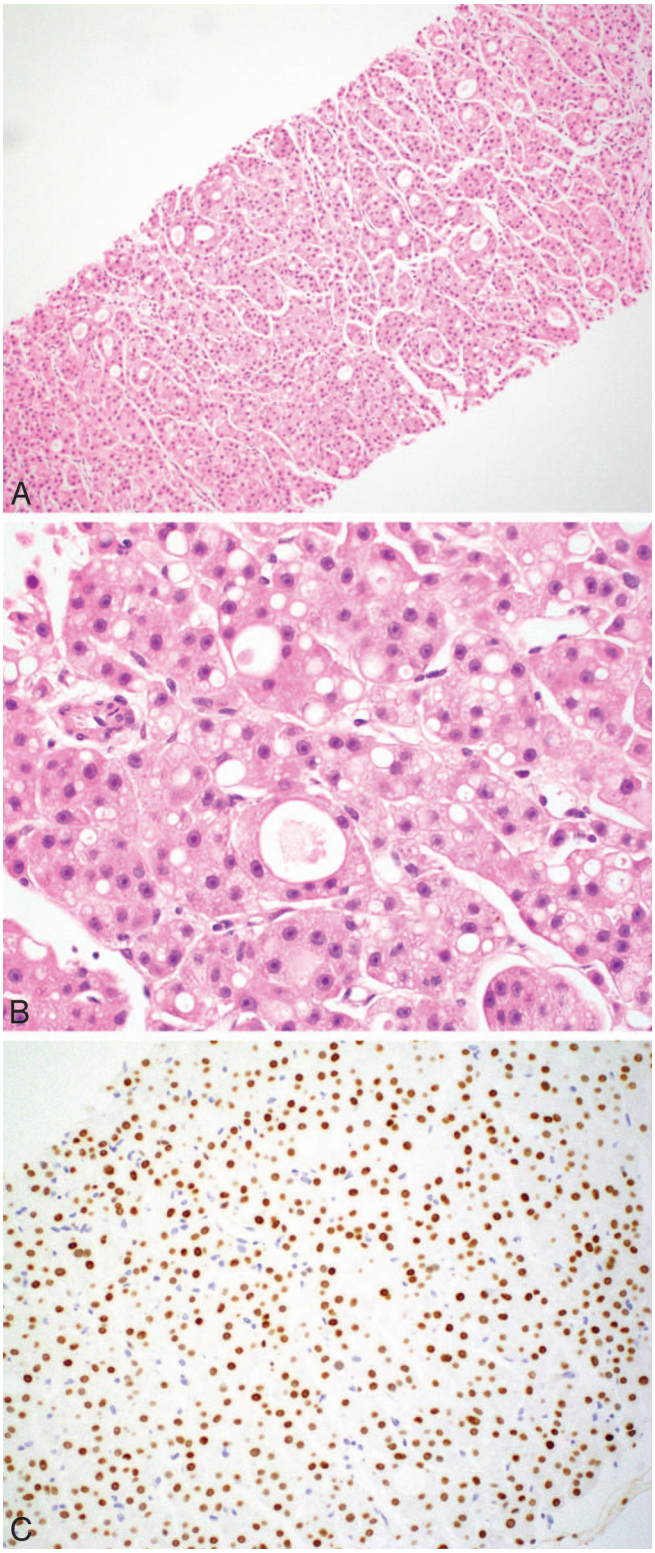

Figure 10. *Metastatic carcinoma in an inguinal lymph node shows a trabecular growth pattern with frequent acinar structures and no intervening stroma (A). Tumor cells have abundant eosinophilic cytoplasm containing fat vacuoles, highly resembling hepatocellular carcinoma (B). Tumor cells are diffusely positive for GATA3 (C; hematoxylin-eosin, original magnifications ×100 [A] and ×400 [B]; original magnification ×200 [C].*

Table 6. Useful Immunomarkers for Pancreaticobiliary Adenocarcinomas					
	Adenocarcinoma				
Marker	Pancreas, %	Gallbladder, %	Extrahepatic Bile Duct, %	Intrahepatic Cholangiocarcinoma, %	Benign Pancreatic and Bile Ducts, %
S100P	90–100	76	76–92	27–48	Negative or focal weak staining
IMP3	71–97	82	50–92	37–90	Negative
Maspin	90–100	59–100	57–91	73–77	Negative or focal weak staining
pVHL[a]	95–100	52–94	93	29	0
SMAD4[a]	60	10	50–55	13–45	0
CK17	60–88	53	59	12–71	0
MUC5AC	67–85	25–82	60–71	12–50	0

[a] Loss of expression.

Immunomarkers to Distinguish Pancreaticobiliary Adenocarcinoma From Benign/Reactive Epithelium

Numerous immunomarkers have been evaluated in this area, as extensively reviewed in a recent publication.[3] To our knowledge, S100P, insulin-like growth factor 2 messenger RNA-binding protein-3 (IMP3), maspin, von Hippel–Lindau gene product (pVHL), SMAD4, CK17, and MUC5AC appear to be better investigated (Table 6), and will be briefly discussed here. Practically, these markers are less than ideal because of their suboptimal sensitivity and/or specificity. Using a panel consisting of a few selected markers (such as IMP3, S100P, maspin, and pVHL) is thus recommended.[162] Ideally, benign epithelial cells show an $S100P^{-}/IMP3^{-}/maspin^{-}/pVHL^{+}$ staining pattern, but malignant cells exhibit an $S100P^{+}/IMP3^{+}/maspin^{+}/pVHL^{-}$ staining pattern.

S100P.—S100P belongs to the S100 protein family originally purified from the human placenta. Its overexpression has been observed in more than 90% of pancreatic ductal adenocarcinomas,[162–167] 76% of gallbladder adenocarcinomas,[168] and 76% to 92% of adenocarcinomas of extrahepatic bile ducts.[165,169,170] Interestingly, the frequency of S100P overexpression is much lower in intrahepatic cholangiocarcinomas, seen in only 27% in one study[167] and 48% in another study.[165] Malignant cells typically show strong nuclear or strong nuclear and cytoplasmic immunoreactivity, whereas benign epithelial cells are either completely nonreactive or show focal/patchy nuclear staining that is weaker in intensity compared with malignant cells (Figure 11, A). Benign epithelial cells do not show as strong cytoplasmic staining as that seen in malignant cells.[171]

IMP3.—A number of human cancers have been shown to overexpress IMP3, which is also known as KOC (K homology domain–containing protein overexpressed in cancers). Cytoplasmic positivity has been demonstrated in 71% to 97% of pancreatic ductal adenocarcinomas in various studies including cytology specimens,[162,167,172–177] 82% of gallbladder adenocarcinomas,[168,178] and 50% to 92% of adenocarcinomas of extrahepatic bile ducts.[169,170,178–180] Intrahepatic cholangiocarcinomas also overexpress IMP3 at a high frequency (82%–90%),[167,181] but lower frequencies (37%–41%) are also reported.[178,182] In our experience, the greatest advantage of IMP3 is its high specificity for malignancy (Figure 11, B). Nonneoplastic pancreaticobiliary epithelial cells are always negative. However, IMP3 does not discriminate invasive adenocarcinoma from dysplasia,[169,171,178] and thus careful histologic evaluation is still essential. Another disadvantage is that approximately 50% of malignant cases show only focal positivity, which significantly reduces its sensitivity because there may be only rare malignant cells present in a given biopsy.

Maspin.—Maspin is a tumor suppressor that is overexpressed in more than 90% of pancreatic ductal adenocarcinomas,[162,167,183,184] 59% to 100% of gallbladder adenocarcinomas,[168,185] 57% to 91% of adenocarcinomas of extrahepatic bile ducts,[186,187] and more than 70% of intrahepatic cholangiocarcinomas.[167,188] Malignant cells typically show strong and diffuse nuclear and cytoplasmic

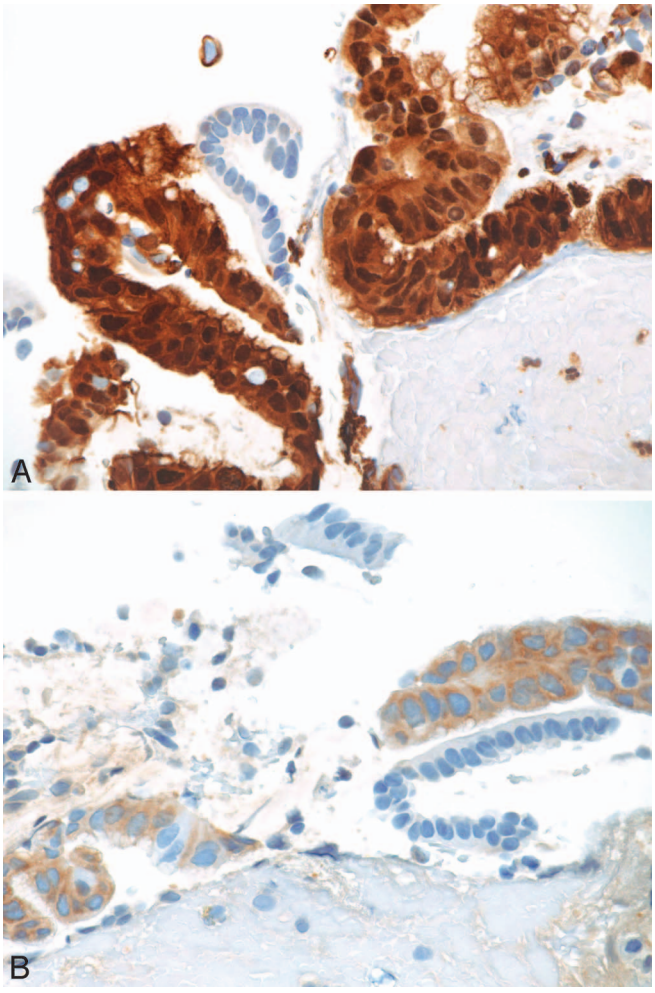

Figure 11. *A bile duct biopsy shows strong nuclear and cytoplasmic positivity for S100P (A) and cytoplasmic staining for IMP3 (B) in suspicious cells, whereas benign biliary epithelium is negative for both markers. Follow-up showed adenocarcinoma of the common bile duct (original magnification ×400).*

immunoreactivity. Benign pancreatic ducts are usually negative for maspin expression, but benign epithelial cells lining the gallbladder and extrahepatic bile ducts have been shown to be positive at a high frequency.[186,187,189] The staining can thus be difficult to interpret despite the observations that positive staining in benign biliary epithelial cells is usually focal/patchy and weaker in intensity in comparison with that seen in malignant cells.

pVHL.—pVHL is normally expressed in benign pancreaticobiliary epithelial cells.[190] Loss of its membranous and cytoplasmic immunoreactivity is seen in more than 95% of pancreatic ductal adenocarcinomas,[162,163,167] 52% to 94% of gallbladder adenocarcinomas,[168,191] and 92.5% of adenocarcinomas of extrahepatic bile ducts,[169] but in only 29% of intrahepatic cholangiocarcinomas.[167] pVHL immunostaining on bile duct biopsies can be difficult to interpret. In one of our studies,[171] reduced immunoreactivity, instead of completely negative staining, was observed in suspicious cells in one-half of malignant cases. The reduction occurs primarily on cell membranes. Therefore, cytoplasmic pVHL staining may be preserved in malignant cells, but the distinctive membranous staining observed in benign biliary epithelium becomes inconspicuous in malignant cells. Comparing suspicious cells with histologically clear-cut benign epithelial cells in the same specimen may help the interpretation. Another problem lies in the lack of homogeneity of immunoreactivity in benign biliary epithelial cells. The staining in these cells is frequently patchy and seldom shows 100% uniformity. It can thus be difficult to determine whether the lack of staining in suspicious cells represents a true loss of immunoreactivity.

SMAD4.—SMAD4, also known as DPC4, is universally expressed in benign pancreatic and bile ducts. Loss of expression is seen in approximately 60% of pancreatic ductal adenocarcinomas, which is associated with poor prognosis.[162,192] SMAD4 loss is also reported in 9.5% of gallbladder adenocarcinomas,[193] 50% to 55% adenocarcinomas of extrahepatic bile ducts,[193,194] and 12.5% to 45% intrahepatic cholangiocarcinomas.[193–195]

CK17.—Cytoplasmic CK17 positivity has been reported in 60% to 88% of pancreatic ductal adenocarcinomas,[162,167,196,197] 53% of gallbladder adenocarcinomas,[198] and 59% of adenocarcinomas of extrahepatic bile ducts.[196] The frequency of CK17 expression in intrahepatic cholangiocarcinomas varies widely, from 12% to 71% in 2 different studies.[167,197] Positive staining can be diffuse or focal, with focal staining seen in up to 40% of cases.[197] Benign epithelial cells lining the pancreatic and bile ducts are typically negative for CK17 expression.

MUC5AC.—Cytoplasmic MUC5AC positivity has been reported in 67% to 85% of pancreatic ductal adenocarcinomas,[36,162,167,197,199] 25% to 82% of gallbladder adenocarcinomas,[199,200] 60% to 71% of adenocarcinomas of extrahepatic bile ducts,[36,199] and 12% to 50% of intrahepatic cholangiocarcinomas.[36,167,197,199] Benign pancreatic and bile ducts are typically negative for MUC5AC expression.

Distinction Between Intrahepatic Cholangiocarcinoma and Hepatic Metastasis of Pancreatic Ductal Adenocarcinoma

The distinction between these 2 types of carcinoma on liver biopsies is essentially impossible histologically but has important clinical implications. Albumin RNA ISH appears to have the greatest potential in this regard. According to a recent study, 82 of 83 intrahepatic cholangiocarcinomas (99%) were positive for albumin RNA. Most of these cases (79%) showed positive staining in more than 50% of tumor cells.[109] All 210 pancreatic adenocarcinomas included in the study were negative for albumin.

We also found a 4-marker panel consisting of S100P, pVHL, CK17, and MUC5AC to be useful.[167] In our study, intrahepatic cholangiocarcinomas less frequently overexpressed S100P, CK17, and MUC5AC, and less frequently showed loss of pVHL expression in comparison with pancreatic ductal adenocarcinomas. An $S100P^{-}/pVHL^{+}/MUC5AC^{-}/CK17^{-}$ staining pattern is essentially indicative of intrahepatic cholangiocarcinoma, whereas the $S100P^{+}/pVHL^{-}/MUC5AC^{+}/CK17^{+}$ and $S100P^{+}/pVHL^{-}/MUC5AC^{-}/CK17^{+}$ staining patterns are suggestive of metastatic pancreatic carcinoma.

Distinction Between Acinar Cell Carcinoma and Other Solid Pancreatic Neoplasms

The diagnosis of acinar cell carcinoma of the pancreas usually needs consideration of differentials from pancreatic neuroendocrine tumor, solid pseudopapillary tumor, and pancreatoblastoma. This is particularly true when diagnostic materials are small biopsies. This topic has been reviewed in detail in a recent publication,[3] and will be only briefly discussed here.

Immunohistochemically, acinar cell carcinomas are positive, with different frequencies, for trypsin, chymotrypsin, BCL10, and lipase. Among these, trypsin and BCL10 appear to be the most sensitive and reliable markers according to a recent study—positive in 95% and 86% of cases, respectively.[201] However, the specificity of BCL10 needs further investigation because weak staining can be rarely seen in neuroendocrine and solid pseudopapillary tumors.[202] Strong staining is also observed in squamous component of adenosquamous carcinomas of the pancreas, although this usually does not create a diagnostic dilemma morphologically. Like trypsin, chymotrypsin is also a specific marker, positive in two-thirds of acinar cell carcinomas. Another specific marker is lipase, but it is positive in only approximately 30% of cases. As mentioned earlier, GPC3 expression has been reported in 25% to 58% of acinar cell carcinomas.[110,121]

In contrast, pancreatic neuroendocrine tumors are positive for the conventional neuroendocrine markers chromogranin, synaptophysin, and CD56. Solid pseudopapillary tumors are typically positive for CD10 and nuclear β-catenin. Therefore, a panel consisting of trypsin, chymotrypsin, chromogranin, synaptophysin, CD10, and β-catenin should be sufficient for the distinction if differential diagnoses include all 3 neoplasms. It is well known, however, that up to 25% of acinar cell carcinomas may also express neuroendocrine markers. Positively stained cells are usually scattered and account for less than 30% of the population. If more than 30% of tumor cells are positive, a diagnosis of mixed acinar-neuroendocrine carcinoma should be considered. Approximately 15% of acinar cell carcinomas may also show positive nuclear staining for β-catenin,[201] which should not be confused with solid pseudopapillary tumor in the presence of positive trypsin staining.

Acinar cell carcinoma accounts for 15% of all exocrine pancreatic neoplasms in children, and thus it needs to be differentiated from pancreatoblastoma. This distinction can be very challenging on biopsy and fine-needle aspiration specimens if the acinar component is prominent. Hopefully, other components, particularly the squamoid nests (or

corpuscles), are also present, which will help in the differential diagnosis. Interestingly, the squamoid nests usually stain for CK19 but usually do not stain for CK7 or squamous marker CK5/6.[203]

Immunomarkers to Help Subclassify Ampullary Adenocarcinoma

Histologic subclassification of ampullary adenocarcinomas into intestinal and pancreaticobiliary types has therapeutic and prognostic significance. Helpful immunomarkers include CDX2, CK17, CK20, MUC1, and MUC2. In an early study, Chu et al[197] demonstrated that a $MUC1^{-}/CK17^{-}/MUC2^{+}/CDX2^{+}$ staining pattern helped identify intestinal type, whereas $MUC1^{+}/CK17^{+}/MUC2^{-}/CDX2^{-}$ pattern helped identify pancreaticobiliary type. A recent study by Ang et al[204] divided ampullary adenocarcinomas into 3 groups based on immunostaining patterns. The intestinal group included those showing positive staining for CK20 or CDX2 or MUC2 and negative staining for MUC1; and those positive for CK20, CDX2, and MUC2 irrespective of MUC1 immunoreactivity. The pancreaticobiliary group included those showing positive staining for MUC1 and negative staining for CDX2 and MUC2 irrespective of CK20. The ambiguous group included those with other combinations of stains, including negative stains for all markers. These observations are further substantiated by more recent studies.[205,206]

Examples

A 65-year-old man with a history of hepatitis B presented with multiple liver lesions. The largest lesion measured 5.4 cm. There was also a nodular lesion in the pancreatic tail, measuring 1.1 cm. A biopsy of the largest liver lesion was performed, which showed an area of tumor cells with acinar formation (Figure 12, A and B). The tumor cells had relatively uniform, round, medium-sized nuclei and moderate amounts of amphophilic cytoplasm. Nucleoli were small but recognizable. Occasional mitotic figures were seen. The nonneoplastic liver parenchyma showed no significant steatosis or fibrosis. No ground-glass hepatocytes were identified. The histologic differentials included metastatic acinar cell carcinoma and neuroendocrine tumor. A panel of immunostains was performed, which demonstrated tumor cells to be positive for trypsin (Figure 12, C), chymotrypsin, and CAM5.2. Tumor cells were negative for chromogranin, Arg-1, and AE1/AE3. Only rare tumor cells were positive for synaptophysin. The findings were supportive of the diagnosis of metastatic acinar cell carcinoma from the pancreas.

IMMUNOMARKERS FOR GI AND PANCREATIC NEUROENDOCRINE NEOPLASMS

Neuroendocrine neoplasms encompass both well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs), both of which can present as a metastasis before the primary tumor is detected. When histologic features suggestive of neuroendocrine differentiation are recognized, immunohistochemical confirmation is almost always sought. Chromogranin is the most specific marker, but its expression frequency varies based on anatomic location: up to 80% in NETs of the pancreas and GI tract proximal to the colon, but only 40% to 60% in those of the colorectum.[207–209] Synaptophysin is less specific but has a higher sensitivity that approaches 95% to 100%.[209] Although CD56 is also highly sensitive for NETs, it suffers from low specificity because it is widely expressed in a variety of tumors without neuroendocrine differentiation. In the setting of NEC, the sensitivity of chromogranin and synaptophysin is much lower.

Grading Neuroendocrine Neoplasms by Ki-67

Gastrointestinal and pancreatic neuroendocrine neoplasms are graded based on mitotic count and Ki-67 proliferation index (Table 7). For mitotic count, it is recommended to count 50 high-power fields (×40) in areas with the highest mitotic activity and express the value as number of mitoses per 10 high-power fields. For Ki-67 index, it is recommended to count 500 to 2000 tumor cells in areas with the highest nuclear labeling ("hot spot") and express the value as percentage. When discordance occurs, the higher grade should be assigned. According to the current classification, both low-grade (grade 1) and intermediate-grade (grade 2) neoplasms are in the same category of well-differentiated neuroendocrine tumor. The high-grade (grade 3) category includes poorly differentiated NECs, such as small cell carcinoma and large cell neuroendocrine carcinoma, as well as tumors that show well-differentiated morphology with grade 2 mitotic count but with grade 3 Ki-67 index (>20%). Whether this latter group should be lumped together with poorly differentiated NEC is a topic of debate. Recent studies have shown a significantly longer survival for these patients in comparison with patients with true poorly differentiated NEC, although their clinical outcome is slightly worse than those with grade 2 NET.[210,211] These data thus suggest that grade 3 neuroendocrine neoplasms are heterogeneous. Tumors with well-differentiated morphology but with grade 3 Ki-67 proliferation index should be separated from true poorly differentiated NECs, and they may be more appropriately designated as "well-differentiated NET with an elevated proliferation rate."[210] Nevertheless, Ki-67 proliferation index has important therapeutic and prognostic implications. Data from a study on a large number of patients with GI NEC (grade 3) have shown less responsiveness to platinum-based chemotherapy but longer survival in patients with Ki-67 index less than 55%.[212]

Immunomarkers to Help Distinguish Among GI, Pancreatic, and Pulmonary NETs

In the setting of metastatic well-differentiated NET of unknown primary, identification of tumor origin has therapeutic implications due to differing response rates to cytotoxic chemotherapy agents and targeted biologic agents. This effort is less important for poorly differentiated NECs because they are all treated with platinum-based chemotherapy regardless of site of origin. A number of immunomarkers have been investigated for their value in the distinction among GI, pancreatic, and pulmonary NETs, which have been comprehensively reviewed in recent publications.[3,213,214] Only the ones that we believe to have potential value to help identify the site of origin are briefly discussed here (Table 8).

TTF-1.—TTF-1 is entirely specific for pulmonary origin, as has been demonstrated by numerous studies. Its sensitivity for pulmonary NETs (typical and atypical carcinoids) ranges from 28% to 77%.[215–221] It essentially excludes other sites of origin if positive in a well-differentiated NET. It should be mentioned here, however, that some small cell carcinomas of nonpulmonary origin can be positive for TTF-1.

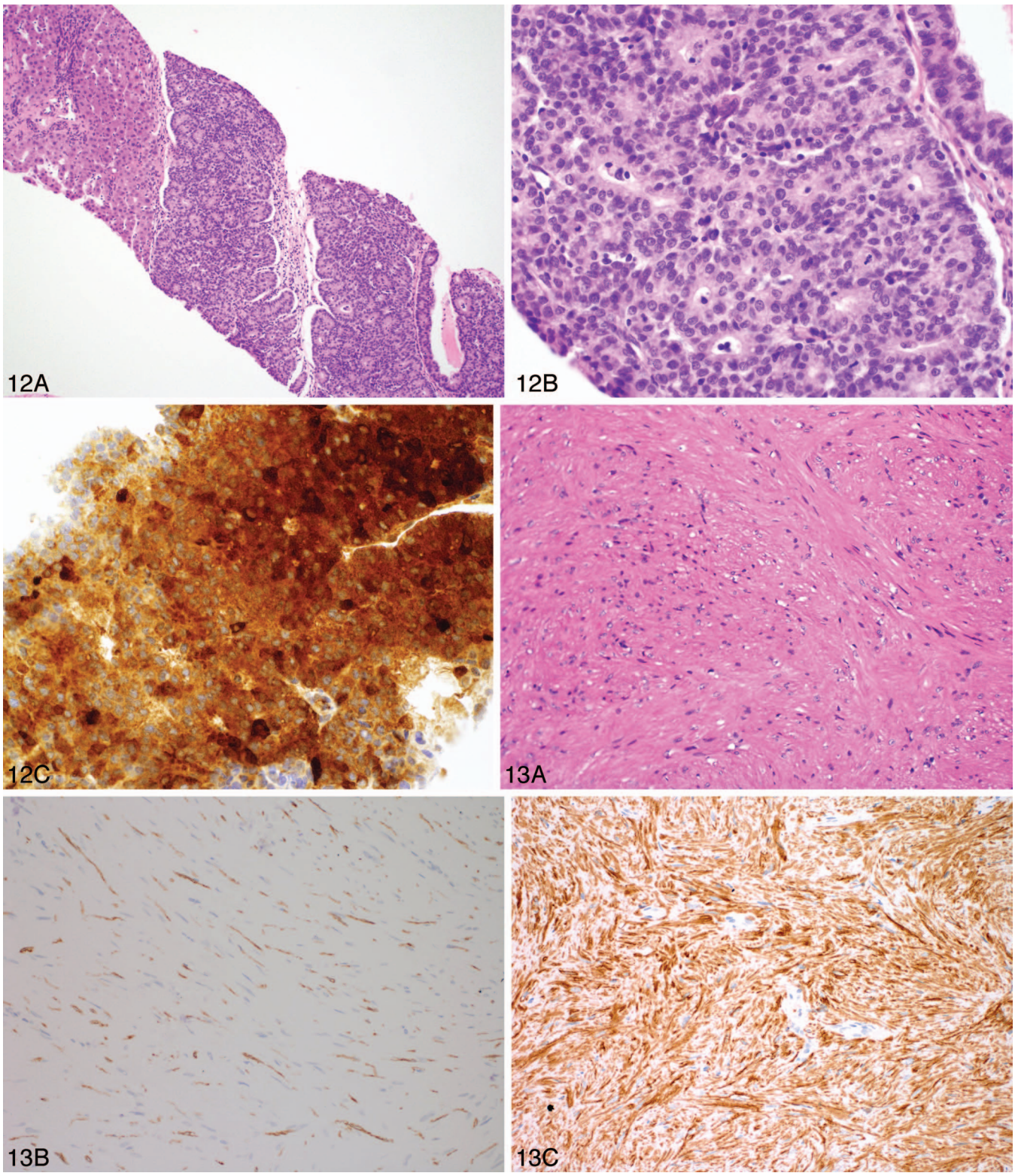

Figure 12. *A liver biopsy shows an area of tumor cells with acinar formation (A). Tumor cells have round, uniform nuclei, small nucleoli, and amphophilic cytoplasm (B). Tumor cells are strongly positive for trypsin (C; hematoxylin-eosin, original magnifications ×100 [A] and ×400 [B]; original magnification ×400 [C]).*

Figure 13. *An esophageal leiomyoma removed by endoscopic resection (A) shows numerous colonized interstitial cells of Cajal within the tumor, as demonstrated by immunostaining for CD117 (B). Similar findings are also seen for DOG1. Note the negative staining in spindle cells. The diagnosis is confirmed by diffuse positive immunostaining for desmin (C; hematoxylin-eosin, original magnification ×200 [A]; original magnification ×200 [B and C]).*

Table 7. Grading of Gastrointestinal and Pancreatic Neuroendocrine Neoplasms		
Grade	Mitotic Count per 10 HPF	Ki-67 Proliferation Index, %
Low grade (grade 1 or G1)	<2	<3
Intermediate grade (grade 2 or G2)	2–20	3–20
High grade (grade 3 or G3)	>20	>20

Abbreviation: HPF, high-power field.

CDX2.—CDX2 is a highly sensitive marker for ileal and appendiceal NETs (midgut origin), expressed in 83% to 100% of cases in most studies.[216–219,221–223] It is also expressed in gastric, duodenal, and colonic NETs at lower frequencies.[216,221–223] Interestingly, rectal NETs appear to show CDX2 positivity less frequently than their colonic counterparts.[217,222,223] CDX2 is infrequently expressed in pancreatic NETs, seen in 0% to 30% of cases in various studies.[216–218,221–224] It is negative in pulmonary NETs in most studies,[218,221,224] but a low frequency of expression (20%) has also been observed.[223] Overall, CDX2 is regarded as a relatively specific marker for GI NETs, particularly those of midgut origin.

SATB2.—A recent study has examined SATB2 expression in well-differentiated NETs, which demonstrated its expression in 90% of hindgut (rectal and sigmoid) NETs.[225] It was detected in 12% of midgut-derived tumors (jejunum, 0%; ileum, 8%; appendix, 25%; cecum and ascending colon, 20%) and in 17% of foregut-derived tumors (stomach, 9%; duodenum, 8%; pancreas, 15%; lung, 23%; thymus, 50%). Data from Geisinger Medical Center have also shown positive SATB2 immunostaining in most NETs from the appendix, colon, and rectum, but rarely seen in NETs from the stomach, duodenum, pancreas, and lung.[2] These observations suggest that SATB2 may serve as a useful immunomarker for hindgut- and midgut-derived NETs.

CDH17.—Two studies have shown CDH17 expression in 92% to 100% of small intestinal NETs and 100% of appendiceal and rectal NETs, respectively.[48,221] The respective frequencies of CDH17 expression were 40% and 12% in pancreatic NETs, and 8% and 24% in pulmonary NETs. Another study showed an overall frequency of 51% in pancreatic NETs, but positively stained cases were of predominantly sclerosing variant (93% versus 36% in nonsclerosing variant).[226] A very limited number of gastric, duodenal, and colonic NETs were also examined in one study,[221] which showed low frequencies of expression ranging from 20% to 50%.

Islet-1.—Islet-1 is a transcription factor involved in the embryogenesis of islets of Langerhans. Studies have shown a high frequency of nuclear expression in pancreatic NETs, seen in 69% to 90% of cases.[223,224,227,228] However, Islet-1 is also detected in 78% to 89% of duodenal and 78% to 100% of rectal NETs.[223,224,228,229] In addition, Islet-1 expression is observed in gastric, ileal, appendiceal, and colonic NETs, but with much lower frequencies. Positive staining is uncommon in pulmonary NETs, seen in 0% to 16% of cases.

PAX8.—Using polyclonal anti-PAX8 antibodies, nuclear immunoreactivity is demonstrated in 67% to 88% of pancreatic, 67% to 100% of duodenal, and 79% to 85% of rectal NETs.[223,224,229–231] It is positive in 7% to 22% of gastric, appendiceal, and colonic NETs, and has been consistently negative in jejunoileal NETs. The reported frequency of positivity in pulmonary NETs ranges from 0% to 23%. Interestingly, positive staining is believed to result from cross-reaction to PAX6 antigen.[232] Therefore, polyclonal antibodies should be used if PAX8 is included in the workup panel and monoclonal anti-PAX8 antibodies will not work. In fact, Lai et al[231] have shown similarly high detection rates for pancreatic, duodenal and rectal NETs when a monoclonal anti-PAX6 antibody is used.

IMMUNOMARKERS FOR GI MESENCHYMAL TUMORS

Relative to epithelial neoplasms, benign and malignant mesenchymal tumors are uncommon in the GI tract.[233,234] Examples include lipoma, leiomyoma, granular cell tumor, schwannoma, gangliocytic paraganglioma, glomus tumor, inflammatory myofibroblastic tumor, plexiform fibromyxoma (plexiform angiomyxoid myofibroblastic tumor), leiomyosarcoma, angiosarcoma, Kaposi sarcoma, and synovial sarcoma, which are immunophenotypically similar to their counterparts in other locations. Undoubtedly, GI stromal tumor (GIST) is the most common mesenchymal tumor in the GI tract[235]; it occurs almost exclusively in this location.

Gastrointestinal clear cell sarcoma–like tumor is a very rare aggressive sarcoma that shares the same translocation involving *EWSR1* in most cases with clear cell sarcoma of soft tissue (formerly known as malignant melanoma of soft parts). However, this malignancy appears to be histologically and immunophenotypically different from clear cell sarcoma, and a new name "malignant GI neuroectodermal tumor (GNET)" is thus recommended.[236]

Table 8. Useful Immunomarkers for Site of Origin of Metastatic Well-Differentiated Neuroendocrine Tumors (NETs)		
Marker	Likely Site of Origin	Comment
TTF-1	Lung	Relatively low sensitivity. No expression in NETs derived from the GI tract or pancreas
CDX2	Small intestine, appendix	Also expressed at lower frequencies in NETs derived from other parts of the GI tract, infrequently expressed in pancreatic NETs, only rarely expressed in pulmonary NETs
SATB2	Rectum, colon, appendix	Limited data. Also expressed at lower frequencies in NETs derived from other parts of the GI tract, pancreas, and lung
CDH17	Small intestine, appendix, rectum	Limited data. Also expressed at lower frequencies in NETs derived from other parts of the GI tract, pancreas, and lung
Islet-1	Pancreas, duodenum, rectum	Also expressed at low frequencies in NETs derived from other parts of the GI tract, uncommon in pulmonary NETs
PAX8 (polyclonal)	Pancreas, duodenum, rectum	Also expressed at low frequencies in NETs derived from other parts of GI tract except the jejunum and ileum, uncommon in pulmonary NETs

Abbreviation: GI, gastrointestinal.

General Immunomarkers for GIST

Gastrointestinal stromal tumor is immunophenotypically related to interstitial cells of Cajal, the pacemaker cells of the GI tract. The most useful diagnostic immunomarkers are CD117 (c-kit) and DOG1 (discovered on GIST 1), which are both positive in more than 90% of cases.[237] Positive stains are typically strong, diffuse, and cytoplasmic. Membranous, perinuclear dotlike, focal, or weak immunoreactivity has also been observed in some cases, particularly those with an epithelioid morphology. Approximately half or more than half of CD117$^-$ GISTs are positive for DOG1, and vice versa.[237,238]

It is well known that CD117 is not GIST specific and can be detected in a wide range of neoplastic and nonneoplastic processes. DOG1 is relatively more specific, but its expression is also observed in some non-GIST neoplasms.[237,239] The vast majority of CD117$^+$ or DOG1$^+$ neoplasms would not cause diagnostic problems because they can be easily separated from GIST histologically. One of the spindle cell neoplasms, intra-abdominal fibromatosis (desmoid tumor), may occasionally enter the differential diagnosis,[240] although CD117 positivity in these lesions is generally believed to be nonspecific, caused by using different antibodies and/or inappropriate antibody dilution.[241] However, desmoid tumors typically show nuclear β-catenin staining, which is absent in GISTs, and negative staining for DOG1.[242] It is interesting to note that both CD117 and DOG1 are expressed in solid pseudopapillary neoplasms of the pancreas at high frequencies, which is not associated with *KIT* or *PDGFRA* mutations.[243,244]

A diagnostic pitfall is that CD117$^+$ and DOG1$^+$ cells are frequently present in leiomyomas of the GI tract (Figure 13, A and B). This is thought to result from colonization and hyperplasia of nonneoplastic interstitial cells of Cajal, which should not be mistaken for GISTs.[237,245,246] The correct diagnosis of leiomyoma relies on typical histologic features, strong and diffuse desmin immunoreactivity (Figure 13, C), and lack of CD117 and DOG1 stains in most spindle cells.

Gastrointestinal stromal tumors are known to be positive for CD34 in approximately 70% of cases. Up to 30% of GISTs may show positivity for smooth muscle actin, but less than 10% express desmin. Positive S100 staining is seen in less than 10% of cases. Another potentially useful marker is protein kinase C-θ (PKCθ), which is positive in more than 80% of GISTs. In comparison with DOG1, PKCθ appears less specific for GISTs, but it may be useful when both CD117 and DOG1 are negative.[238,247,248]

Succinate Dehydrogenase–Deficient GIST

Succinate dehydrogenase (SDH)–deficient GIST is a distinctive clinical and molecular variant arising in the setting of loss of function of the SDH enzyme complex secondary to gene mutations or epigenetic silencing.[249] It is not driven by *KIT* or *PDGFRA* mutations, and it is exclusively seen in the stomach, accounting for 7.5% of gastric GISTs. Most pediatric GISTs and all GISTs associated with Carney-Stratakis syndrome and Carney triad belong to this variant. SDH-deficient GISTs tend to be multinodular, with a plexiform growth pattern and an epithelioid morphology.[249,250] Loss of SDH subunit B (SDHB) expression, as assessed by immunohistochemistry, is the hallmark of this variant. These tumors are also positive for CD117, DOG1, and CD34. Compared with conventional GISTs, SDH-deficient GISTs are more likely to metastasize to lymph nodes and be insensitive to tyrosine kinase inhibitors, but they often follow an indolent or slowly progressive clinical course.

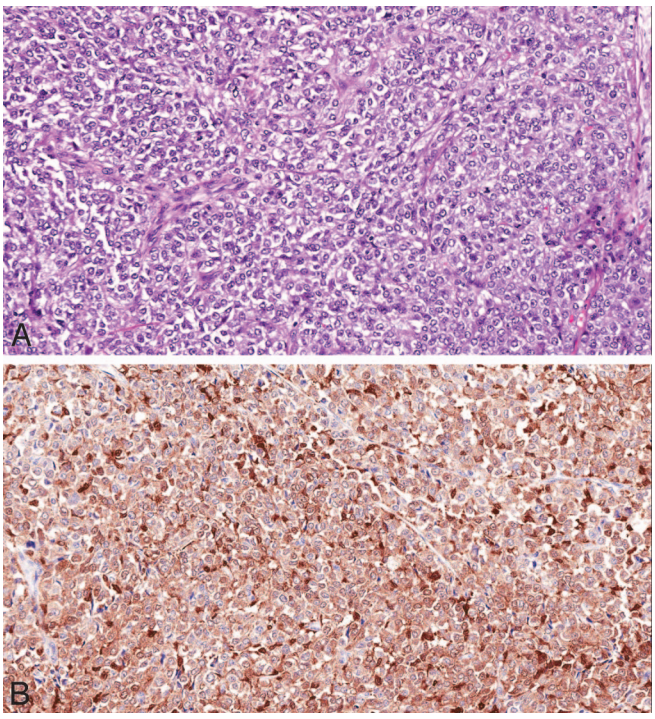

Figure 14. *A case of malignant gastrointestinal neuroectodermal tumor resected from the terminal ileum shows sheets of epithelioid cells with round or ovoid nuclei, indistinct or small nucleoli, and eosinophilic cytoplasm (A). Tumor cells are diffusely positive for S100 (B). Melanocytic markers are negative in this case (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×200 [B]).*

Immunomarkers for GNET

GNET is more frequently seen in the small intestine. Histologically, tumor cells are epithelioid-appearing, with round, ovoid, or spindled nuclei; vesicular chromatin; indistinct to prominent nucleoli; and variable amounts of eosinophilic cytoplasm, which may form diffuse sheets or display nested, alveolar, and microcystic growth patterns (Figure 14, A). Focal areas with cytoplasmic clearing may be seen. Oncocytic cytoplasm has also been reported in 1 case, which can be confused with granular cell tumor on biopsy.[251] Mitoses are frequent, and necrosis is common. Scattered osteoclast-like giant cells can be seen in some cases. Immunohistochemically, tumor cells are consistently positive for S100 (Figure 14, B), SOX10, and vimentin, but negative for melanocytic markers HMB-45, Melan-A, tyrosinase, and MITF (microphthalmia transcription factor).[236,252] Tumor cells are also negative for CD117, DOG1, CD34, smooth muscle actin, desmin, CD99, GFAP, and epithelial markers. Neuroendocrine markers, such as synaptophysin, chromogranin, CD56, and neuron-specific enolase, are variably expressed, seen in 45% to 70% of cases. The Ki-67 proliferation index ranges 22% to 34%.

IMMUNOHISTOCHEMISTRY FOR PROGRAMMED DEATH RECEPTOR-1/PROGRAMMED DEATH LIGAND-1–BASED IMMUNOTHERAPIES

Programmed death receptor-1 (PD-1) is an immune-inhibitory receptor expressed on the surface of lymphocytes,

natural killer cells, dendritic cells, and macrophages. It interacts with two ligands, programmed death ligand-1 (PD-L1) and PD-L2, to function as a key immune checkpoint by inhibiting T-cell activation and cytokine production. PD-L1, also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1), is expressed in various types of tumor cells, and blockage of PD-1/PD-L1 interaction may subject tumor cells to attack by cytotoxic T cells.[253,254] Several PD-1/PD-L1–based immune-checkpoint inhibitors (humanized antibodies), such as atezolizumab, nivolumab, and pembrolizumab, were recently approved by the US Food and Drug Administration for the treatment of advanced melanoma, non–small cell lung cancer, head and neck squamous cell carcinoma, and urothelial carcinoma.[255]

PD-1/PD-L1–based immunotherapies have not been well investigated for tumors of the GI tract, liver, and pancreaticobiliary tract,[256,257] but phase 1 trials have shown no objective responses in patients with CRCs and pancreatic cancers.[258,259] However, a more recent phase II trial that included 41 patients with progressive metastatic carcinomas (11 MMR-deficient CRCs, 21 MMR-proficient CRCs, and 9 MMR-deficient non-CRC carcinomas) showed that MMR-deficient CRCs were more responsive to PD-1 blockage induced by pembrolizumab than MMR-proficient malignancies.[260] Based on data from 149 patients, the US Food and Drug Administration granted accelerated approval on May 23, 2017, to pembrolizumab (KEYTRUDA, Merck & Co, Kenilworth, New Jersey) for adult and pediatric patients with unresectable or metastatic MSI-high or MMR-deficient solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options, or those with MSI-high or MMR-deficient CRC that has progressed following treatment with fluoropyrimidine, oxaliplatin, and irinotecan.[261] Oncologists may occasionally request immunohistochemical testing for PD-L1 expression to determine whether a patient would be likely to benefit from these therapies as the last options. Currently, most institutions send unstained slides to reference laboratories for the staining, and the results are interpreted by pathologists in the laboratories. There is no universally accepted standard for quantitating PD-L1 expression.[253,254] In general, the expression level is measured by the percentage of tumor cells (and/or tumor-infiltrating immune cells) that are positively stained for PD-L1, but different scoring criteria are designed for different inhibitors. For example, the staining results are interpreted as no expression in tumor cells (<1%), any expression (1%–100%), and high expression (50%–100%) for pembrolizumab therapy.

CONCLUSIONS

With continuous discoveries, a large volume of knowledge has been accumulated in the field of immunohistochemistry for neoplastic diseases of the GI tract, liver, biliary tract, and pancreas. Useful immunomarkers are available not only to help practicing pathologists make accurate diagnoses on a daily basis, but also to help guide clinical management of patients. Pathologists will continue to play a critical role in the discovery, validation, and application of new biomarkers, which will ultimately improve patient care.

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