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Immunohistochemistry and Gastrointestinal Carcinomas

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Gastrointestinal (GI) Pathology

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In the past 25 years, the technique of immunohistochemistry has become a near-routine procedure in the assessment of many malignancies involving the GI tract. This technique is useful in various determinations, not the least of which is the origin of a malignancy in the setting of metastatic disease. The following is an effort to summarize the more useful aspects of that collective knowledge.*

Overview of Colorectal Cancer

Incidence

In both men and women, colorectal cancer is the third most commonly diagnosed cancer in the United States (excluding skin cancers). Colorectal cancer incidence rates have been decreasing for most of the past two decades (from 66.3 cases per 100,000 persons in 1985 to 45.5 cases in 2006). This has been attributed to increases in the use of colorectal screening tests that allow the detection and removal of colorectal polyps before the progress to cancer. In contrast to overall declines, among adults younger than 50 years, for whom screening is not recommended for those at average risk, colorectal cancer incidence rates have been increasing by about 2% per year since 1994 in both men and women.[1]

Deaths

An estimated 49,500 deaths from colorectal cancer are expected in 2016 in the U.S. Mortality rates for colorectal cancer have declined in both men and women over the past few decades, with steeper declines in the most recent time period. This decrease reflects declining incidence rates and improvements in early detection and treatment.[1]

Signs and Symptoms

Early stage colorectal cancer does not usually have symptoms; therefore, screening is often necessary to detect colorectal cancer in its early stages. Advanced disease may cause rectal bleeding, blood in the stool, a change in bowel habits, and cramping pain in the lower abdomen.[1]

Immunohistochemistry and Colon Cancer

Immunohistochemical applications surrounding colon cancer are seen at several levels such as: characterization of the tumor (endocrine or epithelial type), hereditary disposition, and for prognostic purposes. The more prevalent use of IHC is in the presence of possible or suspected metastatic disease in which the colon is a possible primary. The common locations for metastases from colon cancers are the liver and lung; both organs of which can produce cancer morphology essentially identical to metastases from the colon. IHC, in a Class I regulations, is used after the primary diagnosis of the tumor has been identified through histopathology and not intended to be reported to clinicians as independent findings.

Metastatic Carcinoma

The most common use of immunohistochemistry in the study of liver tumors is to identify the site of origin of a metastatic tumor when the primary site has not yet been identified. The development and implementation of a panel of immunostains can help resolve almost all diagnostic problems.[2-6] Cytokeratin (CK) 7 and CK 20 are the first step in the immunohistochemical identification of many tumors, and with additional immunostains, some relatively specific for tumors of males and of females, one is able to identify potential sites of origin.[3]

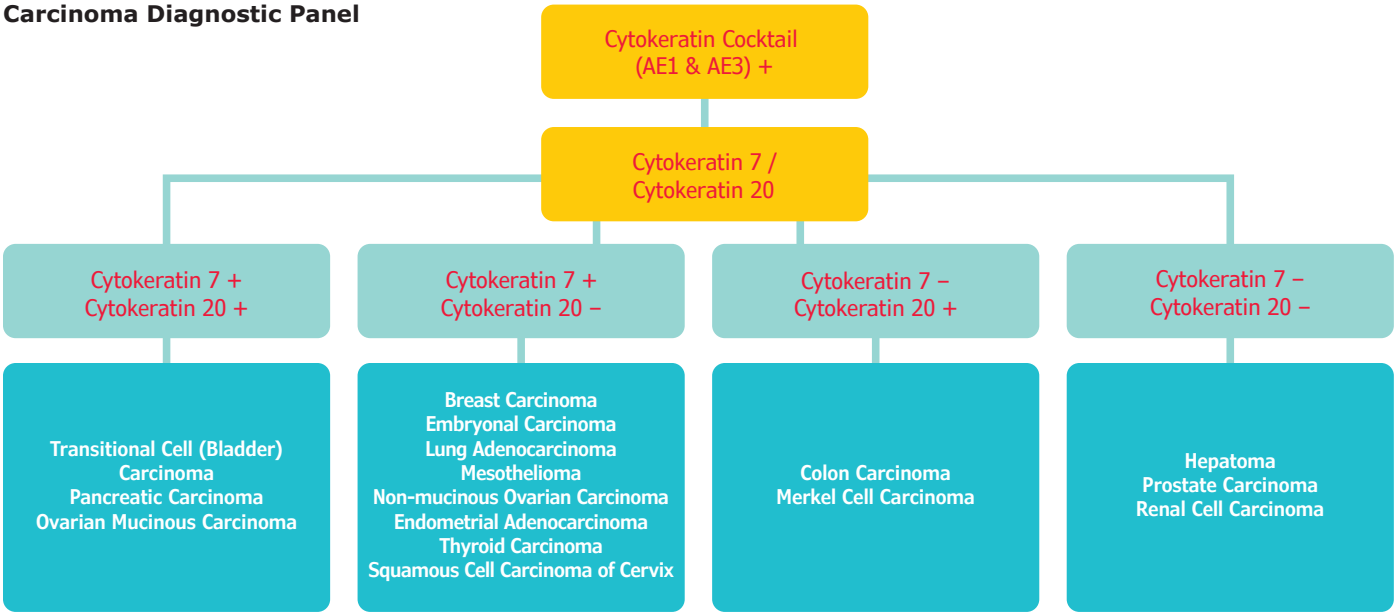
Stomach

Immunohistochemical studies are generally not needed for the evaluation of benign and malignant epithelial tumors of the stomach because the histopathology is generally diagnostic but are used in the study of metastatic gastric carcinoma when the site of origin is not clear or when the macroscopic/radiologic appearance of the tumor is confusing (eg, gastric carcinoma directly and massively invading the liver and histologically indistinguishable from cholangiocarcinoma). In addition, immunohistochemistry is useful for the identification of some variants of gastric carcinomas including hepatoid adenocarcinomas in which hepatostic differentiation can be confirmed by positivity for alpha feto-protein (AFP).[7]

Gastrointestinal Tract Adenocarcinomas VS. Other

	CK 7	CK 20	SATB2	CDX-2	Cadher-in-17	Hep Par-1	Napsin A	P504s	GATA3	S100P
Colorectal Adenocarcinoma	-	+	+	+	+	-	-	-	-	-
Gastric Adenocarcinoma	+/-	+/-	-	+	-	-	-	-	-	-
Esophageal Adenocarcinoma	+/-	+/-	-/+	+/-	+	-	-	-	-	-
Gastrointestinal Stromal Tumor	-	-	-	-	-	-	-	-	-	-
Pancreatic Ductal Adenocarcinoma	+	+	-	-/+	-	-	-	-	-	+
Hepatocellular Carcinoma	-	-	-	-	-	+	-	-	-	-
Lung Adenocarcinoma	+	-	-	-		-	+	-	-	-
Breast Carcinoma	+	-	-	-	-	-	-	-	+	-
Prostatic Adenocarcinoma	-	-	-	-	-	-	-	+	-	-
Urothelial Carcinoma	+	+	-	-	-	-	-	-	+	+
Ovarian Carcinoma	-/+	+/-	-	-	-	-	-	-	-	-

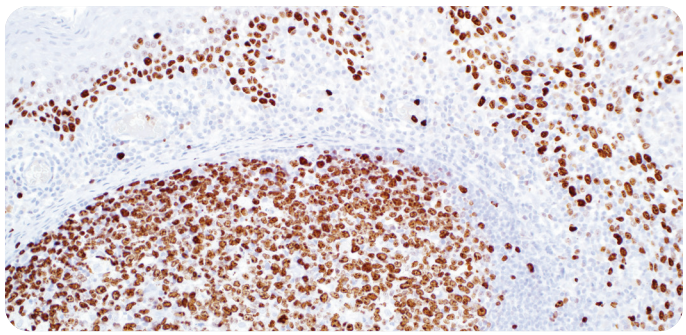
Carcinoma Diagnostic Panel



Adenocarcinoma
Gastric adenocarcinomas will react with many antibodies directed against keratins, including AE1 & AE3, CK 35betaH11, CK 18, CK 19, CK 7, and CK 20. When CK 7 and CK 20 are used together, many gastric adenocarcinomas will stain with both CK 7 and CK 20.[5,8-10] Approximately 25% will be positive for one and negative for the other (eg, CK 7+/CK 20-, CK 7-/CK 20+), and a small number of cases will be negative for both. CDX-2, initially thought to be specific for colon carcinoma, will be reactive in more than 50% of cases[11-12] and may be indicative of a lesser degree of invasiveness.[13-14] Even Hep Par-1, a useful marker for hepatocytes, will be positive in more than 50% of gastric cancers, including signet-ring cell carcinoma.[15] The quantity and quality of mucus production by gastric carcinoma, as evaluated by immunohistochemical study of mucins, may be prognostically important; MUC2 expression is associated with poor survival.[16]

Neuroendocrine Carcinoma
Neuroendocrine carcinoma characteristically stains with synaptophysin, chromogranin, villin, and CD57.[17-18] In contrast to gastric adenocarcinoma, carcinomas occurring in the second part of the duodenum may be negative for both synaptophysin and chromogranin but will often react with somatostatin. The proliferation marker Ki-67 and the adhesion molecule E-cadherin have been used to assess aggressiveness of neuroendocrine carcinoma.[19] An increased Ki-67 proliferation index (< or =2%, 3-20% and >20% for G1, G2, and G3 lesions respectively)[20] predicts aggressive behavior, and loss of E-cadherin may predict lymph node metastasis.

Gastrointestinal Adenocarcinoma with Neuroendocrine Differentiation
Gastric adenocarcinomas, both intestinal type and signet- ring cell type, can have neuroendocrine differentiation that may not be obvious with hematoxylin-eosin staining but will show staining with chromogranin and/or synaptophysin.[21]



Ki-67

Gastrointestinal Stromal Tumors

CD117 stains most cases of gastrointestinal stromal tumor, including metastases.[21-25] Although there may sometimes be variation in distribution of CD117 positivity within a given tumor, in most cases staining is diffuse. When CD117 is positive in tumors other than gastrointestinal stromal tumor, the staining is almost always patchy. CD34 staining can also be seen in gastrointestinal stromal tumor. 10 to 15% of GIST are negative for CD117. DOG1 antibody has been shown to label the majority of CD117 negative GISTs.[26] In addition, loss of SDHB has been proven to be consistent feature of SDH-deficient GISTs, thereby being helpful for the identification of this disease variant.[27]

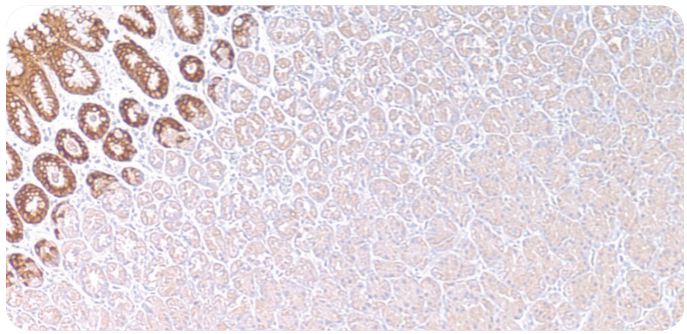
GIST Mutation vs. Wild Type

	CD34	CD117	DOG1
GIST, Kit Mutation	+	+	+
GIST, PDGFRA Mutation	-	-	+
GIST, Wild Type	+/-	+	+

Colon

Adenomas and Adenocarcinoma
Adenomas (tubular adenoma, tubulovillous adenoma, villous adenoma) demonstrate the same immunohistochemical reactions as colonic adenocarcinoma. Almost all react with antibody directed against CK 20 and a minority will also stain focally with CK 7, in contrast to pancreatic adenocarcinomas, most of which are CK 20 negative and CK 7 positive. Differentiation of metastatic colorectal adenocarcinoma from adenocarcinoma arising at other sites can sometimes be challenging. Pulmonary adenocarcinoma can resemble colorectal adenocarcinoma. CK 7 and CK 20 can be helpful in this regard, with CK 7 usually strongly positive in lung adenocarcinomas and CK 20 usually negative; the reverse pattern is seen with colorectal adenocarcinoma. In addition, thyroid transcription factor 1 (TTF-1) is generally positive in lung cancers, and CDX-2 and beta-catenin are generally positive in colorectal cancers. Endometrioid-type carcinomas can also be histologically indistinguishable from colorectal carcinoma. Here, again, CK 7 is positive in almost all endometrioid adenocarcinomas and only mildly reactive in colorectal adenocarcinomas. CK 20 is generally negative for lung primaries but positive for colorectal.

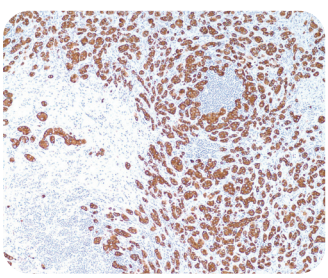
Appendiceal Adenocarcinoma
Appendiceal adenocarcinoma will typically show staining for MUC5AC, in contrast to colonic adenocarcinoma in which this antibody is rarely reactive.[28-29] This is particularly useful in studying mucinous adenocarcinomas that have metastasized in the abdomen. Beta-Catenin is another differentiating antibody, positive in almost all colonic adenocarcinomas and negative in appendiceal adenocarcinomas. In women with abdominal mucinous carcinomatosis, distinction of colonic and appendiceal adenocarcinoma from ovarian adenocarcinoma is important. In colonic tumors, both villin and beta-catenin are often expressed; in appendiceal metastases villin is often expressed, but beta-catenin is unusual and in ovarian mucinous adenocarcinomas neither villin nor beta-catenin are seen.[30] Similar to appendiceal lesions, ovarian carcinomas express MUC5AC and similar to colorectal adenocarcinoma, ovarian mucinous adenocarcinomas express CDX-2. Useful supplements to the basic panels for unknown primaries in which appendiceal or ovarian mucinous tumors are suspected are MUC5AC and beta-catenin. Villin can also be helpful because it typically has a ‘‘brush-border’’ pattern of staining in both colonic and appendiceal adenocarcinoma and is typically cytoplasmic in ovarian and pancreatic lesions.[31]



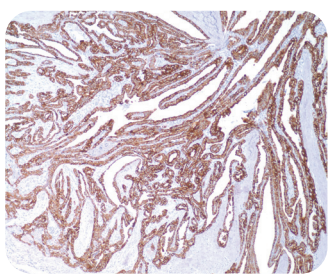
MUC5AC

Pancreas

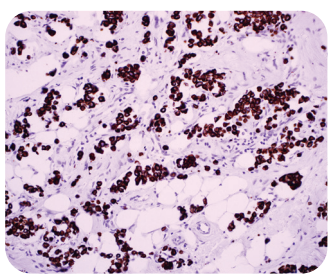
Invasive Ductal Adenocarcinoma
Adenocarcinoma of the pancreas usually derives from precursor stages of pancreatic ductal dysplasia. The immunostaining pattern of high-grade pancreatic intraepithelial neoplasia is the same as that of invasive pancreatic adenocarcinoma and cannot be used to differentiate between them. Pancreatic ductal adenocarcinomas resemble adenocarcinomas of the bile ducts and gallbladder in their light microscopy appearances and also in their immunophenotypical presentations. Pancreatic adenocarcinomas react with a variety of keratin antibodies, including CK 8, CK 17, CK 18, CK 19, CAM 5.2, and AE1 & AE3.[5,32] Pancreatic adenocarcinoma is generally both CK 7 and CK 20 positive. Pancreatic adenocarcinoma can also be faintly CDX-2 positive.[4,11] Almost all pancreaticobiliary adenocarcinomas are CEA positive and CA-125 positive. They may also have a minor component



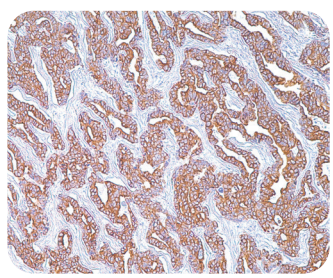
Ki-67



Cytokeratin 19



Cytokeratin

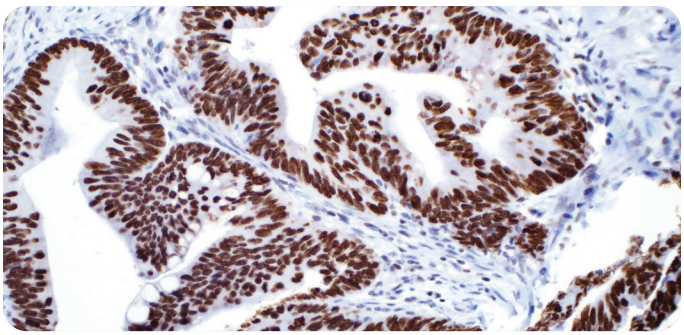


Cytokeratin Cocktail

of neuroendocrine cells, which will react with somatostatin, synaptophysin, chromogranin, or other neuroendocrine markers.[33] Therapy outcomes can also be predicted with positive vascular endothelial growth factor and negative SMAD4 (DPC4) immunostaining.[34] Loss of expression of SMAD4 has also been shown in bile duct epithelium in cases of chronic gallstone disease.[35] Well-differentiated metastatic pancreatic carcinoma to the liver may be difficult to distinguish from benign bile duct lesions in biopsy material. Unlike the benign lesions, however, they typically express p53, cytoplasmic mCEA, and other markers including CA-125.[36]

Neuroendocrine and Endocrine Cell Tumors, Low Grade and High Grade

Low- and high-grade neuroendocrine tumors tend to show similar immunophenotypic expressions, but, in general, the intensity of staining is less with high-grade tumors. They can be grouped by the predominant secreted hormone (eg, somatostatin, gastrin) but usually also stain with synaptophysin and chromogranin, as well as with various keratins, including CK 8, CK 18, and CK 35betaH11. CK 7 and CK 20 are generally negative.[37-41] CD56 and CD57 tend to stain more intensely in high-grade neuroendocrine tumors than in low-grade, in a membranous pattern; CD56 will also be positive in a variety of other tumors.[37-42] Serotonin can also be demonstrated.[43] High-grade neuroendocrine tumors can also stain with calcitonin, and metastatic lesions can be misinterpreted as having arisen in the thyroid.[41] Epithelial cytoplasmic expression of CD10, in contrast to membrane staining, is more commonly seen in malignant than in benign pancreatic endocrine tumors.[44]



SATB2

SATB2

Special AT-rich sequence-binding protein 2 (SATB2) is a recently described marker that functions as a nuclear matrix-associated transcription factor. It has been reported that SATB2, in combination with CK20, could identify almost all colorectal carcinomas,[45] including poorly differentiated colorectal carcinomas.[46] Upper gastrointestinal (GI) carcinomas and pancreatic ductal carcinomas are usually negative for SATB2,[45] and ovarian carcinomas, lung adenocarcinomas, and adenocarcinomas from other origin are rarely positive for SATB2.[45-46] Therefore, SATB2 is a good marker for identifying a carcinoma of colorectal origin when working on a tumor of unknown primary.[45-47] Another potential utility of SATB2 is to identify neuroendocrine neoplasms/carcinomas of the left colon and rectum because SATB2 is usually negative in other neuroendocrine neoplasms of the GI tract, pancreas, and lung.[48]

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34385 Ver. 1.0 MS_FL6072EN 12/2020