Esophagus

Squamous cell carcinoma (SCC)

SCC of the esophagus has been associated with various geographic, ethnic and lifestyle risk factors. Compared to adenocarcinoma of the esophagus which is the more common tumor in the United States, SCC is much more common in Asian countries, where up to 40% have been linked to HPV infection (1). SCC is more common in males, particularly African American males and lifestyle risk factors such as smoking and alcohol are believed to increase the risk of SCC up to 90% (1,2). Patients may present with dysphagia, odynophagia and weight loss. Although SCC can develop in any part of the esophagus but are more commonly found in the middle and lower third portions of the esophagus (3,4). On gross examination the tumor is usually circumferential with sharp margin and are often ulcerate. Polypoid forms may also be seen (1). Microscopically, the tumors resemble their counterparts in the skin and show varying degrees of squamous differentiation with extensive keratinization in the well-differentiated forms and lack of cohesiveness, with even a pseudoglandular configuration in poorly-differentiated forms. The immunohistochemical profile of SCC is similar to that of its skin counterpart: CK7−, CD20−, CK5/6+, CK10+ and CK14+ (Figure 1A). SCC is always positive for p63 (Figure 1B) (5-9). Additionally, most cases of esophageal SCC are also positive for p53, a finding not seen in normal esophageal mucosa (8). As mentioned before, HPV has been found to be associated with esophageal SCC, particularly in cases reported from China (10) and Africa (11) where up to 20-40% of esophageal cases have been shown to be positive for HPV, particularly type 11, 16 and 18. Many of these HPV cases have been found to be positive for p16 as well, much similar to cases of cervical SCC (12,13).

Intestinal metaplasia(IM)

IM (Barrett’s esophagus) is defined as the presence of specialized intestinal epithelium in the distal esophagus above the level of the lower esophageal sphincter (14,15), and according to the American College of Gastroenterology Barrett’s mucosa is defined as a change in the esophageal epithelium of any length that can be recognized by endoscopy and is confirmed to be intestinal metaplasia (IM) by biopsy. Most patients with IM are adults, although this condition may develop in children with gastroesophageal reflux and following chemotherapy (16,17). Gastroesophageal reflux is believed to play a role in IM as up to 10% of patients with IM suffer from reflux. The importance of IM lies mainly in its association with the development of adenocarcinoma since more than 80% of patients with adenocarcinoma have been shown to have associated IM. Histologically, IM is quite similar to normal small intestinal mucosa with the presence of absorptive cells,
goblet cells and Paneth cells. IM is further classified into three categories based on the degree of dysplasia: negative for dysplasia, indefinite for dysplasia and positive for dysplasia (low-grade and high grade). These are based on evaluation of surface maturation in comparison to underlying glands, architecture of the glands, cytologic features, inflammation and the presence of erosions/ulcers (18). In addition to its unique morphologic features, IM also shows a unique immunohistochemical profile. Greater than 95% cases of IM have characteristic superficial CK20 staining pattern along with a strong superficial and deep CK7 staining (19-21). Unlike normal gastric mucosa where cells are positive for MUC1, MUC5AC and MUC6 but negative for MUC2 the cells in IM/Barrett’s esophagus are positive for MUC2. The intensity of MUC2 staining varies according to the number of goblet cells, being higher in complete IM and lower in incomplete IM. Other monoclonal antibodies which are specific to gastric or colonic mucosa have also been used to confirm the diagnosis of IM such as Das-1 antibody which binds to colonic epithelial protein in absorptive cells, and HepPar-1 which is expressed in small intestinal mucosa but not normal gastric and colonic mucosa. Another marker that has been found to be useful in distinguishing the degree of dysplasia is AMACAR. AMACAR is a marker for prostate adenocarcinoma, and is also expressed in normal small intestinal and colorectal mucosa. AMCAR has also been found to be expressed in cases of IM with dysplasia, with an incidence of 20%, 40% and 80% in cases of indefinite, low grade and high-grade dysplasia, respectively (22) but is negative in cases of IM without dysplasia (22,23). p53 expression can also aid in the classification of dysplasia as up to 60% of cases of high-grade dysplasia and carcinoma express p53 in comparison to just 30% of cases classified as indefinite for dysplasia and low grade dysplasia (24,25). The increase in p53 expression is accompanied by increased Ki-67 labeling (26,27). IM with and without dysplasia can also be separated by using a combination of the markers described above. IM without dysplasia is usually positive for HepPar-1 and MUC2 and negative for AMCAR, whereas IM with dysplasia and adenocarcinoma often express AMCAR but not HepPar-1 or MUC2 (28,29).

Esophageal adenocarcinoma is rapidly increasing in incidence in the United States (30,31). Predisposing factors include male gender, white race, obesity, Barrett’s esophagus, smoking and alcohol consumption (32). Most cases of esophageal adenocarcinoma involve the lower one third of the esophagus and show glandular differentiation. These tumors usually express CK7, variable CK20, AMACAR, and weak focal CDX-2, an immunohistochemical pattern similar to that of gastric adenocarcinoma. P16 is negative in esophageal adenocarcinoma unlike SCC (26).

**Stomach**

**Gastric epithelial dysplasia (GED)**

GED most commonly occurs in men in their fifth to seventh decades, and is more common in westernized countries. There is often no gross features which can be recognized endoscopically but microscopically there may be glandular crowding, branching, budding, cytologic atypia, decreased apical mucin and frequent mitoses. GED may arise in either native gastric or intestinalized gastric epithelia, and is divided into three categories: indefinite for dysplasia, low-grade and high-grade dysplasia (33). Studies have show that 15% of low-grade dysplasia may show progression to carcinoma while cases of carcinoma...
from high-grade dysplasia is seen in 80-85% (34,35). Immunohistochemical stains may assist in the assessment of dysplasia as p53 expression and Ki-67 positive dysplastic cells also increase as dysplasia increases (36).

Gastric intestinal metaplasia (GIM)

GIM is similar histologically and immunohistochemically to Barrett’s esophagus/esophageal adenocarcinoma. It is defined as the development of goblet and/or Paneth cells within the normal gastric mucosa. The two main types of GIM are the complete type (type I) characterized by its resemblance to normal small intestinal mucosa with absorptive cells, Paneth cells and goblet cells; and the incomplete type (types II and III) where there are columnar and goblet cells. Most cases of gastric carcinoma arise within areas of incomplete GIM, and show CK7 and CK20 in the superficial and deep crypt cells (37-39). GIM is also positive for HepPar-14 and CDX-2 (40). The complete type of GIM is negative for MUC1 and MUC5AC but positive for MUC2 while incomplete GIM is positive for both (22). *H. pylori* infection has been found in over 80% of patients with GIM (37) which can then be identified by using the Das-1 antibody which stains *H. pylori* in gastric associated GIM (36).

Gastric adenocarcinoma (GA)

GA is the second most common cancer worldwide with the highest rates in Asia. It is more common in males and has been associated with risk factors such as low socioeconomic status, cigarette smoking, nitrites, chronic gastritis and *H. pylori* (41-43). The majority of gastric adenocarcinomas is located in the pylorus and antrum (50-60%), followed by the cardia (25%), and the body or fundus (15-25%) and may be exophytic, flat or ulcerated. There are two classifications of GA, the intestinal type, which has well-formed glands lined by columnar to cuboidal epithelial cells (Figure 2), and the diffuse type which shows single to poorly

![Figure 2](image-url)
formed nests of cells growing in an infiltrate pattern (signet ring cell carcinoma) (Figure 3A) (43,44). Intestinal type GA shows variable expression of CK7 (Figure 2B), CK20 (Figure 2C), CDX-2 (Figure 2D), MUC1, and MUC5AC (45-47). Diffuse type of GA usually develops de novo, and is not associated with H. pylori induced IM. Over 70% of cases of the diffuse type of GA are positive for CDX-2 (Figure 3B), CK7 (Figure 3C), HepPar-1 (Figure 3D) and variable expression of CK20 (Figure 3E), MUC2 and MUC5AC, but negative for MUC1 and E-cadherin (Figure 3F) (48,49). Cases of poorly differentiated adenocarcinoma with prominent lymphoplasmacytic stroma may also be positive for EBV (50,51).

Tumors of the upper gastrointestinal tract such as Barrett’s esophagus, esophageal adenocarcinoma and gastric adenocarcinoma may show similar immunohistochemical findings, Table 1 compares each of their unique immunohistochemical profile (52,53).

**Gastrointestinal stromal tumor (GIST)**

Stromal tumors comprise the majority of primary

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**Table 1** Comparison of immunohistochemical profiles of Barrett’s, esophageal and gastric adenocarcinoma

<table>
<thead>
<tr>
<th>Immunochemical markers</th>
<th>Barrett’s esophagus without dysplasia</th>
<th>Barrett’s esophagus with dysplasia</th>
<th>Esophageal adenocarcinoma</th>
<th>Gastric adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMACR</td>
<td>0%</td>
<td>98%</td>
<td>73%</td>
<td>/</td>
</tr>
<tr>
<td>HepPar-1</td>
<td></td>
<td>97%</td>
<td>13%</td>
<td>31%</td>
</tr>
<tr>
<td>Keratin 7</td>
<td></td>
<td>97%</td>
<td>94%</td>
<td>51%</td>
</tr>
<tr>
<td>Keratin 20</td>
<td></td>
<td>95%</td>
<td>45%</td>
<td>48%</td>
</tr>
<tr>
<td>MUC1</td>
<td>40%</td>
<td>23%</td>
<td>47%</td>
<td>31%</td>
</tr>
<tr>
<td>MUC2</td>
<td>95%</td>
<td>95%</td>
<td>0%</td>
<td>29%</td>
</tr>
<tr>
<td>CDX-2</td>
<td>77%</td>
<td>37%</td>
<td>46%</td>
<td>60%</td>
</tr>
<tr>
<td>P53</td>
<td>0%</td>
<td>75%</td>
<td>57%</td>
<td>/</td>
</tr>
<tr>
<td>Ki-67</td>
<td>11%</td>
<td>85%</td>
<td>76%</td>
<td>/</td>
</tr>
</tbody>
</table>

Reference: (52)
nonepithelial neoplasms in the stomach, and GIST is the
most common GI mesenchymal neoplasm. GIST may occur
anywhere within the GI tract but is most common in the
stomach (60%) (53), with prognosis varying according to
their location (54). Histologically, GISTs resemble smooth
muscle tumors with spindle or epithelioid cells. Gastric
GIST generally have a better prognosis compared to small
intestinal GIST and may have either a predominantly
spindle cell pattern or an epithelioid pattern. Features
associated with a more aggressive behavior include a high
mitotic rate (>5/per 50 hpf), large size (>5 cm), invasion,
location within the fundus or gastrointestinal junction,
coagulative necrosis, ulceration and epithelioid morphology
(55,56). The vast majority of GISTs show a diffuse
cytoplasmic staining with membranous accentuation of
CD117 (KIT) (Figure 4A). CD117 is the product of the
c-kit gene and is a type-3 tyrosine kinase receptor which
is normally expressed in the interstitial cells of Cajal, mast
cells, melanocytes, fetal endothelial cells and CD34-positive
hematopoietic stem cells. CD117 is also positive in a variety of
tumors such as mastocytoma, seminoma, pulmonary small cell
carcinoma and blastic types of myeloid sarcoma just to name a
few (57). Although CD117 positivity is present in most GIST,
it is not required for diagnosis (58), since 5-10% of gastric
GIST and 4% of small intestinal GIST may be negative for
CD117 (57). Most CD117 negative GISTs are positive for
another GIST marker-DOG-1 (Figure 4B). The diagnosis
of GIST then requires examination of the morphologic,
immunohistochemical and molecular PDGRFRA mutation
analysis. Other immunohistochemical markers which may be
positive in GIST include PDGFRα5, CD34 (80%),
SMA (20%) (55), DOG1 (79%), and CK18 (59). Antibody
cocktails for keratin such as AE1/AE3 are generally negative
in gastric GIST as they are negative for CK7, CK17, CK19
and CK20. S-100 is also only positive in <1% of gastric
GISTs (57). GFAP is negative in GIST and thus helps in
differentiating from gastrointestinal schwannoma which is
GFAP positive.

Extranodal marginal zone lymphoma of mucosa-
associated lymphoid tissue (MALT)

MALT is the most common type of lymphoma to occur
in the stomach (60). Development of MALT has been
associated with Helicobacter pylori infection with induction
of remission reported by antibiotic treatment of the H.
pylori (61). The lymphoma cells are B-cells and infiltrate
the marginal zone around the preserved follicles. The cells
are small to medium in size with a monocytoid appearance.
Plasmacytic differentiation is often present in gastric
MALT lymphomas (60). Tumor cells are positive for CD20,
CD79a and Pax-5 but negative for CD5, CD10, and CD23.
Aberrant CD5 co-expression has been described while
co-expression of CD43 has been reported in one-third
of cases (62). Cytogenetic abnormalities in MALT include
t[11;18], t[1;14], t[14;18] and t[3;14] with t[11;18] being the
most common translocation in MALT lymphomas involving
the stomach (63,64).

Small intestine

The small intestine includes the duodenum, jejunum and
ileum extending from the pylorus to ileocecal valve, yet
neoplasms in the small intestine are extremely rare. Global
incidence of small intestinal neoplasms is less than 1.0
per 100,000, and in the United States they represent only
0.4% of total cancers (65). The different types of primary small intestinal tumors include adenocarcinomas, carcinoid tumors, lymphomas and sarcomas (66,67).

Adenocarcinoma of the small intestine

Adenocarcinoma of the small intestine is the most common type of primary malignancy in the small bowel, and generally presents in older males with a higher incidence in African Americans than Caucasians. Most cases are sporadic but reported risk factors include sporadic adenomatous polyps, familial adenomatous polyposis and Crohn’s disease. Presentation may include obstruction, jaundice, GI bleeding and abdominal pain, and often presents at an advanced stage. The most common locations for adenocarcinoma are the duodenum and proximal jejunum. Adenocarcinomas may present as polypoid, infiltrative or constricting lesions, with tumors in the duodenal and ampullary regions generally being exophytic in nature (67). Histologically, these tumors are similar to colorectal adenocarcinomas and are characterized by the degree of pleomorphism, complex glandular architecture, luminal necrosis and invasion. Small intestinal adenocarcinomas are CK7+ in more than half of all cases (Figure 5A), unlike normal small intestinal mucosa which is CK7- and colorectal adenocarcinomas which are CK7-/CK20+ (68). Adenocarcinomas of the small bowel are also positive for CK20 (Figure 5B), CDX-2 (Figure 5C), and villin (68).

Adenocarcinoma of ampulla of Vater

Adenocarcinoma of ampulla of Vater comprise about 5-6% of cancers arising (69) in the head of the pancreas. These tumors cause obstruction of the bile duct even at a very small size and hence patients often present early in the disease course with jaundice. Two major histologic types have been described: an intestinal type, arising from the overlying intestinal mucosa of the papilla (intestinal type adenocarcinoma of duodenal papillary origin) and a pancreatobiliary type, derived from the ductal epithelium which penetrates the duodenal muscularis propria (ampullary carcinoma of pancreatobiliary origin) (69). The intestinal type adenocarcinoma is much more common and has a much better prognosis (70), hence it is important to differentiate these two entities. Fortunately, the immunophenotype of these two types differ, with the intestinal type adenocarcinoma of duodenal papillary origin being positive for CK7, CK20, MUC2 and CDX-2 but negative for MUC1, MUC5AC and CK17; whereas ampullary carcinoma of pancreatobiliary origin is positive for MUC1, CK7, and CK17 but negative for MUC2 (69,70).

Gastrointestinal neuroendocrine tumors

Gastrointestinal neuroendocrine tumors are tumor derived from endocrine cells and can arise anywhere in the gastrointestinal tract. Most neuroendocrine tumors and carcinomas (carcinoids) in the GI tract are well differentiated. The location of these carcinoid tumors can be divided based on their embryologic derivation into carcinoids of the foregut (esophagus, stomach and duodenum), midgut (jejunum, ileum, appendix and ascending colon) and hindgut (transverse colon, descending colon, sigmoid and rectum) (71). Tumors from each different region of the gastrointestinal tract may secrete different hormones as well. Foregut and midgut carcinoid often produce serotonin and substance P while hindgut carcinoids may produce glucagon like peptide, pancreatic polypeptide, and polypeptide YY (72-78). In spite of these differences, these tumors share similar morphologic features such as clusters/sheets/nests of neuroendocrine cells with round to ovoid nuclei, “salt and pepper” chromatin and moderate amounts of clear cytoplasm. All gastrointestinal neuroendocrine tumors are positive for the generic markers of neuroendocrine differentiation such as chromogranin A, synaptophysin and NSE, as well as PGP 9.5, and CD56 (79). Determining the origin of the tumor may be challenging; however, immunohistochemical stains can be very helpful.

Figure 5 Immunohistochemical features of small intestinal adenocarcinoma. A. CK7 positivity; B. CK20; C. CDX-2 showing diffuse positivity
Carcinoids from the foregut and midgut are generally positive for chromogranin A and CD56, while those from the hindgut are usually negative (73,80,81). Hindgut carcinoids on the other hand often express prostatic acid phosphatase (82). A less helpful marker is CDX-2, which although positive for most colorectal carcinomas has an immunoreactivity of about 40% in well differentiated carcinoids (83-87) but has a reported 80% expression rate in poorly differentiated carcinoids (80).

Carcinoid tumors make up about a third of the neoplasms in the small intestine. They most often occur in the ileum and rarely in the duodenum and can be separated by their location: duodenal and jejunoileal carcinoids. Duodenal carcinoids, similar to any carcinoid in the gastrointestinal tract can be further divided by the type of cells which make up the tumor into gastrinomas (G-cell tumors), somatostatinomas (D-cell tumors) and a small percentage of the undefined type (88). Classification of neuroendocrine tumors is based on the degree of differentiation. Most carcinoids are well-differentiated carcinoid (50-75%), well-differentiated neuroendocrine carcinoma and poorly differentiated neuroendocrine carcinoma (<1-3%) (88). Carcinoid tumors usually show a monotonous proliferation of small bland polygonal cells with round nuclei, “salt and pepper” chromatin and moderate amounts of cytoplasm in either a nested (type A), trabecular (type B) or acinar (type C) pattern. Distinction between benign and malignant carcinoid is based on the presence or absence of metastasis rather than just on histology.

**Colon and rectum**

**Colorectal cancer (CRC)**

CRC is the third most common cancer diagnosed in the United States and third most common cause of cancer deaths. Risk for development of colorectal carcinoma increases significantly after the age of 40. In addition to age, lifestyle modifiers and genetic risk factors all play a role in CRC. Family history and genetics plays an important role as well, particularly in patients less than 50 years. Approximately 25% of CRC arise in patients with a family history of disease while 5% arise in the setting of an established familial syndrome (89). The genetic syndromes associated with CRC can be divided into the hereditary polyposis colon cancers (HPCC) and hereditary nonpolyposis colon cancers (HNPPC). Categories of HPCC include: (I) Familial adenomatous polyposis; (II) MUTYH-associated polyposis; (III) hyperplastic polyposis syndrome; (IV) Peutz-Jeghers syndrome; and (V) Juvenile polyposis syndrome (89). Of the polyposis CRC familial adenomatous polyposis (FAP) is the most common. FAP is an autosomal dominant disease with 100% penetrance. Patients with FAP develop hundreds to thousands of adenomatous colonic polyps starting in the second decade of life with a 100% risk of CRC (89,90). Another category of HPCC is the MUTYH - associated polyposis, an autosomal recessive colon cancer syndrome which accounts for 0.5% to 1% of all CRC (91,92). Patients with MUTYH - associated polyposis may have zero to thousands of polyps like FAP, with an estimated lifetime risk of CRC around 80% (92). Hyperplastic polyposis syndrome (HPS) is characterized by the development of numerous, large hyperplastic and sessile serrated polyps, with a 35% to 54% prevalence of CRC development (93). Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant disease characterized by the development of pigmented macules on lips, mucosa, hands and feet, along with development of hamartomatous polyps as well as cancers in the CRC, stomach, small bowel, pancreas, breast, sex cord, uterus, cervix and skin. Patients with PJS have a 39% lifetime risk of CRC and 93% risk for any other malignancy (94). Juvenile polyposis syndrome (JPS) typically presents in childhood and has an associated 10-38% lifetime risk of developing colon cancer (95). Lynch syndrome/HNPCC is the most common autosomal dominant inherited colon cancer family syndrome responsible for 10% of colon cancer cases before the age of 50 years (96). The risk of CRC is related to the development of innumerable adenomas. Diagnosis of HNPCC is based on the Amsterdam criteria taking into account the extracolonic malignancies which are common in HNPCC involving the endometrium, stomach, ovary, urinary collecting system, skin, pancreatic and biliary tract (97). Patients with HNPCC have a seven fold increased risk of CRC and present at least 20 years younger than the general population (98).

The histopathologic types of CRC recognized by the World Health Organization include adenocarcinoma, mucinous adenocarcinoma, signet ring carcinoma, small cell carcinoma, adenosquamous carcinoma, squamous cell carcinoma and undifferentiated carcinoma.

**Colorectal adenocarcinoma (CA)**

CA may be polypoid, exophytic, ulcerative, or infiltrative, and can present anywhere within the colon. Polypoid tumors are more common in the cecum and right colon, while ulcerative tumors are more common in the left colon and rectum. Microscopically, CA form glands with mucin (Figure 6A) and are classified as well, moderate or poorly differentiated. In addition to the genetic syndromes discussed previously, sporadic CAs have been associated with mutations in the APC, K-ras and p53. CA is characterized by a CK7 negative and CK20
positive (Figure 6B) immunophenotype, and thus can be differentiated from non-ampullary small intestinal adenocarcinomas by their lack of expression of CK7 and positivity for CK20, Table 2 summarizes several key immunohistochemical stains which can help in distinguishing these two entities.

Appendiceal adenocarcinomas with mucinous differentiation, as well as rectal adenocarcinomas on the other hand may also show expression of CK7 and thus differentiation from metastatic ovarian mucinous tumors is required. Other markers for CA include villin which is positive in 80% of CA (Figure 6C), CDX-2 which is positive (Figure 6D) in almost all well differentiated CA and adenomas but less than 10-20% of poorly differentiated adenocarcinomas may be weakly positive or negative (99). CDX-2 is a transcription factor involved in the proliferation and differentiation of intestinal epithelial cells, and the incidence of CDX-2 expression in adenocarcinoma of the gastrointestinal tract increases from esophagus to rectum and in cases where it is positive all tumor cells show a strong staining pattern. In addition to expression in CA, CDX-2 may also be expressed in ovarian mucinous adenocarcinomas and bladder adenocarcinomas (99-101). CA with mucinous features have an immunophenotype similar to conventional CA with tumor cells positive for CK20, CDX-2, MUC2 (Figure 6E) and β-catenin (102), while the signet ring type of CA is also positive for CDX-2, CK20 and MUC2.

### Table 2

Comparison of immunohistochemical profiles of small intestinal and colorectal adenocarcinoma

<table>
<thead>
<tr>
<th>Markers</th>
<th>Non-ampullary small intestinal adenocarcinomas</th>
<th>Colorectal adenocarcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK7</td>
<td>24/24 (100%)</td>
<td>1/24 (4%)</td>
</tr>
<tr>
<td>CK20</td>
<td>1/23 (4%)</td>
<td>22/23 (96%)</td>
</tr>
<tr>
<td>AMACR</td>
<td>1/24 (4%)</td>
<td>182/266 (68%)</td>
</tr>
<tr>
<td>CDX-2</td>
<td>14/14 (100%)</td>
<td>74/75 (99%)</td>
</tr>
<tr>
<td>Villin</td>
<td>14/14 (100%)</td>
<td>60/75 (80%)</td>
</tr>
</tbody>
</table>

Reference: (52)

**Figure 6** Histologic and immunohistochemical features of colon adenocarcinoma. A. Colon adenocarcinoma; B. Diffuse CK20 positivity in tumor cells; C. Villin shows diffuse positivity; D. CDX-2 is diffusely positive; E. MUC2 positive in mucin producing cells

### Appendix

The appendix may develop any of the tumors described above from the small and large intestines; however, there are a few unique entities at this site including mucinous neoplasms and goblet cell carcinoid tumors.
Goblet cell carcinoid of the appendix (GCC)

GCC is a distinct type of carcinoid tumor within the colorectum which exhibits both neuroendocrine and intestinal-type goblet cell morphology (103). Up to 50% of patients with GCC present with disseminated disease hence it is an important differential to consider particularly in female patients, where an ovarian primary is often one of the first considerations (104). GCC arise from the base of the epithelial crypts of the appendiceal mucosa without associated epithelial dysplasia, and show a submucosal growth pattern with invasion of the appendiceal wall comprised of goblet or signet ring cells in clusters or glands separated by smooth muscle stroma (Figure 7A) (105). The signet ring cells are positive for PAS, mucicarmin, pancytokeratin, CDX-2 (Figure 7B), CK20, MUC2 and CEA; as well as focally positive for chromogranin (Figure 7C) and synaptophysin. Up to 25% of cases are negative for neuroendocrine markers (106,107).

Mucinous neoplasms of the appendix

Mucinous neoplasms of the appendix are the most common type of epithelial neoplasms in the appendix. These neoplasms present in a wide spectrum ranging from mucinous cystadenoma, low-grade mucinous neoplasm, and disseminated peritoneal adenomucinosis or cystadenocarcinoma, mucinous carcinoma, and peritoneal mucinous carcinomatosis (108). These tumors are associated with pseudomyxoma peritonei, a clinical condition of gelatinous ascites, commonly also seen in ovarian mucinous neoplasms (109-112). The classification of mucinous neoplasms within the appendix remains a controversial issue. Broadly speaking, mucinous neoplasms of the appendix can be divided into two major types: those that resemble conventional colonic adenocarcinoma with potential for destructive growth, nodal or solid organ metastasis; and those, which are predominantly low-grade mucinous neoplasms with potential for peritoneal dissemination (108). Their immunophenotype is similar to that of other mucinous tumors in the lower gastrointestinal tract being positive for MUC-2, CK20, CDX-2 and beta-catenin, but with lower expression of CDX-2 and beta-catenin. In addition, mucinous adenocarcinomas of the appendix with positivity for CK7 (113), hence differentiation from upper GI and mucinous neoplasms from other areas is necessary.

Anal tumors

The anal canal is defined as the region located between the junction of the colorectal-type glandular mucosa and the junction between the squamous mucosa lined distal portion. Despite its short length, the anal canal produces a wide variety of tumor types. Tumors within the anal canal include: (I) squamous cell tumors including condyloma acuminatum, flat squamous dysplasia, invasive squamous cell carcinoma and its variants; (II) adenocarcinoma rectal type, anal gland adenocarcinoma, fistula-related mucinous adenocarcinoma and intraepithelial adenocarcinoma (Paget disease); (III) neuroendocrine neoplasms; (IV) melanoma; (V) mesenchymal tumors and (VI) lymphoma.

Squamous cell carcinoma

Squamous cell carcinoma is the most common type of tumor within the anal canal. The incidence of SCC of the anal region is higher in females (114). There is also an increase in incidence in high-risk patient population (HIV positive patients) and an association with HPV (115). Three distinctive subtypes are recognized based on their distinctive histologic features: verrucous carcinoma, squamous cell carcinoma with mucinous microcysts and small cell (anaplastic) carcinoma (116).

Adenocarcinoma of the anal canal

Adenocarcinoma of the anal canal is much less common,
accounting for about 10% of all anal cancers (117). Similar to squamous cell carcinoma of the anal canal, adenocarcinomas in this region have been associated with high-risk HPV types. Other risk factors include inflammatory conditions such as Crohn’s disease and chronic anal fistulas (118).

Of the various types of adenocarcinomas in this region, Paget disease is the one most likely to cause difficulties in diagnosis. Paget disease of the anal canal may arise from an underlying anal gland adenocarcinoma, adnexal (eccrine gland) adenocarcinoma or an underlying visceral malignancy, most commonly a colorectal adenocarcinoma. The use of immunohistochemistry can help differentiate these as those arising from anal gland adenocarcinoma would be CK7+/CK20+/CDX-2+/GCDFP-15- (119,120), from adnexal adenocarcinoma would be CK7+/CK20-/CDX-2-/GCDFP-15+ and that from a colorectal adenocarcinoma would be CK7-/CK20+/CDX-2+/GCDFP-15+ (119-124). These tumors may also need to be differentiated from mammary Paget disease (CK7+/CEA+/EMA+/HER-2/neu+/MUC1+/ER+/CK20-/CDX-2-/GCDFP-15+) (125-133) and Paget disease of the vulva (CK7+/CEA+/EMA+/HER-2/neu+/MUC1+/ER-/CK20-/CDX-2-/GCDFP-15-) (133-136).

Pancreas

Although pancreatic tumors are one of the less common tumors within the gastrointestinal tract, it is the 4th leading cause of cancer mortality in the United States in both men and women (137). Due to the nature of the disease, pancreatic cancers often do not cause symptoms until the later stages. In fact, less than 10% of pancreatic cancers are detected at a stage where cure is possible. The overall survival for this group of cancers is only about 5% (137). Based on the histological features, pancreatic tumors can be divided into three main categories: exocrine neoplasms, neuroendocrine tumors and mixed exocrine-endocrine tumors.

Pancreatic ductal adenocarcinomas

Pancreatic ductal adenocarcinomas make up the majority (>95%) of pancreatic tumors. Pancreatic cancer is more common among the elderly, with a higher incidence in men than in women and more common in blacks compared to other races (137). Risk factors include cigarette smoking, family history, diabetes mellitus and obesity (138). Presentation often occurs late in the disease course as epigastric pain, weight loss, painless jaundice, light clay-colored stools, dark urine, pruritus, and nausea. Pancreatic ductal carcinomas often present as poorly defined masses involving the head of the pancreas (>60%) with variable degrees of necrosis which may lead to the formation of cysts (139). Depending on the degree of differentiation these tumors may show well formed glands in a haphazard pattern (Figure 8A) or individual cells forming sheets, single cell infiltration or poorly formed glands in poorly differentiated adenocarcinoma. Variants of adenocarcinoma included adenosquamous carcinoma, colloid carcinoma, hepatoid carcinoma, medullary carcinoma, signet ring cell carcinoma and undifferentiated carcinoma (139). Most cases show expression of CK7 (Figure 8B), while a subset focally express CK20 (40%) (Figure 8C), a feature which allow for differentiation from extra-pancreatobiliary non-mucinous adenocarcinomas. Pancreatic ductal carcinomas are also positive for CK8, CK17 (Figure 8D), CK18, CK19, CEA, CA19-9, Dupan-2, MUC1, MUC4 and MUC5AC (140-143).

Pancreatic intraepithelial neoplasia (PanIN)

Pancreatic intraepithelial neoplasia (PanIN) has been speculated to be the precursor lesion of pancreatic ductal adenocarcinomas for over fifty years, but it is only recently that its significance and role in pancreatic carcinoma has been established. It is one of three major categories of precursor lesions defined by ongoing epidemiological and molecular studies, the other two being intraductal papillary-mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN) (144). PanIN is the most common and most defined precursor lesion of pancreatic ductal carcinoma (145). These lesions are often found at the same time as the diagnosis for pancreatic adenocarcinoma, and share similar genetic alterations such as K-ras mutation, inactivation of tumor suppressor genes and both show expression of MUC1 and MUC5AC but not MUC2 (143,146,147).

Mucin-producing cystic neoplasms of the pancreas

Mucin-producing cystic neoplasms of the pancreas comprise of two entities: mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN). Mucinous cystic neoplasms occur almost exclusively in perimenopausal women in the body or tail of pancreas. These lesions generally do not show any communication with the pancreatic duct system and often has a thick wall and are multiloculated (148). Histologically, the cyst is lined at least focally by columnar mucinous epithelium and has an ovarian-type stroma (149). These tumors are positive for CK7, CEA, CA19-9, pancytokeratin, MUC-2 (in goblet cells) and EMA (150). Most MCNs also express MUC5AC while MUC-1 is only expressed in invasive MCNs (151). The ovarian-type stroma present in MCN may be positive for ER, PR, inhibin and frequently CD10 (152-154).
Intraductal papillary mucinous neoplasms

Intraductal papillary mucinous neoplasms are more common in older men and are most often located at the head of the pancreas. These lesions comprise of an intraductal proliferation of mucinous epithelium within the main pancreatic and/or the branching ducts (155,156), usually in a papillary arrangement without ovarian-type stroma. The epithelial cells may be of intestinal type, pancreatobiliary type or null type (similar to gastric foveolar epithelium) or morphologically unclassifiable (150). Intestinal-type IPMN often show a colloid-type pattern of invasion and are frequently positive for CDX-2, and MUC2 but negative for MUC1, while the pancreatobiliary type is more aggressive and is negative for CDX-2, MUC2 and positive for MUC1 (150). The null type on the other hand is generally negative for MUC1, CDX-2 and MUC2 (157). Mucinous carcinomas which arise from IMPN are frequently positive for MUC1 but less often positive for MUC5AC (158).

Solid-pseudopapillary neoplasm (SPN)

SPN is an uncommon pancreatic tumor most often found in young women (159). Patients present with nonspecific symptoms related to the intra-abdominal mass such as abdominal pain and early satiety. SPNs are generally large, well circumscribed tumors which can occur anywhere within the pancreas (160). Microscopically, they form dense nests of uniform eosinophilic cells surrounding delicate vasculature resembling ependymal rosettes. The tumor cells often have nuclei with grooves and clear vacuolated cytoplasm (159). Slide preparations from material obtained by fine-needle aspirate biopsy show a distinct “Chinese character-like” appearance due to the branching capillaries are surrounded by small uniform tumor cell and show prominent nuclear grooves and/or inclusions in the tumor cells and background of metachromatic myxoid material (161). The tumor cells are positive for alpha-1-antitrypsin, vimentin, NSE, ER-β, PR,
Serous cystic neoplasms (SCN)

SCN are neoplasms composed of glycogen-rich, ductular-like epithelial cells. Most SCNs are benign while others may be precursors to invasive cancer. Correlation with the patient’s age, gender, relationship between cysts and larger pancreatic ducts, cysts contents (serous fluid, mucin or necrotic debris), lining cell and nature of the stroma are all required in evaluation. Serous cystadenomas are more common in females and often present with nonspecific symptoms such as pain, nausea, weight loss. These tumors are well-circumscribed masses which on sectioning shows innumerable small cysts with a “honeycomb” appearance and often a central scar (167). The cells have a central round to oval nuclei, inconspicuous nucleoli and clear cytoplasm and are positive on periodic acid-Schiff (PAS) stain due to the abundant intracytoplasmic glycogen. SCNs are positive for low molecular weight keratins (CK7, CK8, CK18, and CK19), MUC1, EMA (168), alpha inhibin, NSE, and MUC6 but negative for MUC5A, CK17 and CEA (markers positive in pancreatic ductal adenocarcinoma) as well as chromogranin, synaptophysin, vimentin, PR, and β-catenin (169).

Pancreatic neuroendocrine tumors (PNETs)

Pancreatic neuroendocrine tumors (PNETs) are rare neoplasms with an incidence of 1 per 100,000 individuals per year and comprising just 1-2% of all pancreatic tumors (170). Pancreatic neuroendocrine tumors can present at any age but are most common during the 4th to 6th decades of life with no sex predilection (170). Although most tumors are sporadic there is an association with hereditary endocrinopathies such as multiple endocrine neoplasia type I (MEN I), von Hippel-Lindau syndrome, neurofibromatosis and tuberous sclerosis. PNETs can be broadly divided into functional and nonfunctional tumors. Functional neuroendocrine tumors are tumors which produce a variety of clinical syndromes due to an excess in hormones and include insulinoma, gastrinoma, glucagonoma, VIPoma, and somatostatinoma (171). The non-functional PNETs may also produce hormones but generally do not have symptoms due to the hormone production. These tumors are classified according to the WHO classification into well differentiated endocrine tumor, well differentiated endocrine carcinoma and poorly differentiated endocrine carcinoma based on size, mitotic count, Ki-67 proliferation index, angioinvasion and metastasis. PNETs are diffusely positive for synaptophysin consistently while chromogranin A may show a more focal staining pattern of variable intensity (170). They also express CD56, CD57, PDG 9.5 and NSE (172,173), as well a CK8 and 18. In differentiating PNETs from neuroendocrine tumors from other primary sites, CDX-2 may also be helpful as it has been reported to be positive in 20-30% of PNET cases (83,84). Other markers shown to be positive in pancreatic endocrine tumors include trypsin, chymotrypsin and lipase (174,175).

Pancreatoblastoma

Pancreatoblastoma is the most common pancreatic neoplasm of childhood. Most cases occur in children less than 10 years of age (176), and there is a slight male predominance and association with Beckwith-Weidemann syndrome (177). These tumors are generally large, and may arise in either the head or the tail of the pancreas as well-circumscribed and lobulated masses. Histologically, the tumor has a lobular appearance with well-defined islands of small epithelial cells separated by fibrous bands with a geographic pattern of lighter and darker staining cells due to the different cell types present. The tumor cells in the darker staining areas are small with centrally placed nuclei and prominent nucleoli with scant cytoplasm, while cells in the lighter areas have abundant eosinophilic cytoplasm and may be spindled in shape with a whorling pattern. The presence of occasional squamoid nests is characteristic for this lesion (178). The immunophenotype of the tumor cells often shows acinar differentiation as they are PAS-positive with diastase resistant cytoplasmic granules and positive for trypsin, chymotrypsin and lipase. Despite the presence of squamoid nests, pancreatoblastomas are negative for squamous markers (negative for high molecular weight keratins CK14, CK5/6, and CK17) and CK7 (179) but positive for CK8, CK18, CK19, EMA and cytokeratin and membranous β-catenin (180). Up to half of the tumors may exhibit neuroendocrine differentiation with focal chromogranin and synaptophysin positivity while the cells of ductal differentiation are highlighted by their production of mucin, CEA and CA19 positivity (181). Pancreatoblastomas have also been found to show alterations in the β-catenin/ APC pathway in up to 80% of cases, hence its positivity by immunohistochemistry (180).
Acinar cell carcinoma

Acinar cell carcinoma is more common in adults and presents with non-specific gastrointestinal symptoms such as abdominal pain, nausea and weight loss. Some patients may have subcutaneous fat necrosis and polyarthralgia due to increased levels of serum lipase (159). These tumors are often large and can occur anywhere within the pancreas but are more often found at the head of the pancreas. Microscopically, acinar cell carcinomas show nests of pyramidal cells arranged in solid or acinar patterns. Tumor cells have basally oriented nuclei, single prominent nucleoli and granular cytoplasm. Acinar cell carcinomas are positive for pan-cytokeratin, CK8, CK18, zymogen, trypsin, chymotrypsin and lipase, but negative for CK7 and CK19 (182,183). Scattered cells positive for neuroendocrine markers are present in one-third of cases. A few cases may demonstrate the APC/β-catenin gene mutation (184).

Mixed exocrine-endocrine tumors

Mixed exocrine-endocrine tumors are defined as malignant epithelial neoplasms where the ductal and endocrine cells are intimately mixed in the primary tumor with at least one-third to one-half of tumor cells showing positivity for endocrine markers (185). Ductal differentiation is defined as ductular formation and mucin production (174) and presence of ductal markers like CEA, CA19.9, while ductal acinar cells can be highlighted by pancreatic enzymes like trypsin, chymotrypsin and lipase (186,187). Endocrine cells can be characterized by positivity for endocrine markers chromogranin A and synaptophysin. These mixed tumors generally behave as ductal adenocarcinomas (187). It is important to remember that 40-80% of usual ductal adenocarcinomas may contain endocrine cells, but the metastases from these tumors generally lack endocrine cells (174,187).

Liver

Primary tumors of the liver are divided into epithelial and non-epithelial (mesenchymal) lesions and then further into benign and malignant categories. The majority of the mass lesions within the liver are benign lesions such as focal nodular hyperplasia (FNH), regenerative nodules, adenoma, cirrhosis, and vascular lesions. Of the malignant lesions metastatic tumors are far more common than primary hepatocellular carcinomas.

Hepatocellular carcinoma (HCC)

HCC is the sixth most common malignancy and third most common cause of mortality from cancer worldwide (188). Risk factors for HCC include chronic liver disease such as chronic hepatitis C virus, hepatitis B virus, cirrhosis, obesity related liver disease and alcohol-related liver disease (189). Symptoms of HCC include abdominal pain, fullness, mass or signs and symptoms of cirrhosis, with the most helpful indicator being elevated serum levels of alpha fetoprotein (AFP). On gross examination, HCC presents as a single large mass which may or may not have satellite nodules. Histologically, well-differentiated HCC is difficult to differentiate from normal liver as the polygonal cells resemble hepatocytes and are arranged in trabecular pattern lined by sinusoids mimicking normal liver but have intracytoplasmic bile (Figure 9A) (188). Differentiation between HCC and normal/benign liver is therefore very difficult especially on small needle-core biopsies, and immunohistochemical stains are thus very helpful (189,190). Two immunohistochemical stains that can differentiate HCC from normal/benign liver are Glycan-3 (Figure 9B), a marker which is exclusively expressed in neoplastic processes and not normal tissue in humans (191), and CD34 (Figure 9C) a vascular marker which highlights the increased vascularity seen in HCC (192-196). Another marker which is only expressed in HCC and not normal liver is AFP (Figure 9D) (197). Other markers for HCC include CD10 (Figure 10A), polyclonal CEA (Figure 10B) which highlights the canaliculi (198,199), HepPar-1 (Figure 10C) which reacts with both neoplastic and normal liver tissue. and AFP (200). HCC express only a limited number of keratin markers, namely CK8 and CK18 and thus most metastatic carcinomas can be excluded as they generally express a larger variety of keratin markers such as CK5/6, CK7, CK14 or CK20 in comparison to HCC (201).

Cholangiocarcinoma (CC)

CC make up approximately 3% of all gastrointestinal cancers worldwide (202). These tumors are more common in elderly men and have been associated with cirrhosis, hepatitis C; infections by Clonorchis sinensis and Opisthorchis viverrini, primary sclerosing cholangitis, Thorotrast exposure, genetic hemochromatosis, alpha-1-antitrypsin deficiency and contraceptive steroid use (202-204). CC arises from the intrahepatic bile duct epithelial cells and can be divided based on the location of origin, intrahepatic/ peripheral CC arise at the confluence of the right and left hepatic ducts, while the extrahepatic CC arise between the ampulla of Vater and the hepatic hilium (205). Depending on their location the presenting symptoms may also differ. Histologically, CC is similar to ductal adenocarcinoma of the pancreas with tumor cells arranged in tubules and
Figure 9 Histologic and unique immunohistochemical features of hepatocellular carcinoma. A. Hepatocellular carcinoma; B. Glypican-3 shows diffuse positivity in tumor cells; C. CD34 highlights the increased vascularity within the tumor; D. AFP is aberrantly expressed.

Figure 10 Immunohistochemical features of hepatocellular carcinoma. A. CD10 shows a canicular staining pattern; B. polyclonal CEA also highlights the canaliculi; C. HepPar-1 with diffuse intracytoplasmic granular positivity.
glands which may be cribriform or form nests, solid cords and papillary structures (205). CC is positive for CK7, CK17, mucin, CEA (cytoplasmic and luminal), CAM 5.2, CK19, EMA and CK20 (30-70%) (206).

**Hepatoblastomas (HB)**

HB are the most common primary liver tumors in children, with the majority occurring in children less than 2 years of age (207). These tumors have a slight predominance in males, low-birth weight infants and have been associated with familial adenomatous polyposis, and various chromosomal abnormalities as well as mutations in the β-catenin gene (208). HB generally present as solitary masses in the right lobe of liver and are classified based on their histology into six main patterns: fetal pattern, embryonal pattern, macrotrabecular pattern, small cell undifferentiated pattern, mixed epithelial and mesenchymal pattern and mixed pattern with teratoid features (209). Immunohistochemically HBs are positive for HepPar-1, AFP and EMA while those with the small cell pattern may be positive for cytokeratin with the mesenchymal areas being positive for vimentin (210).

**Gallbladder**

Benign bile duct proliferations: Benign bile duct proliferations such as bile duct hamartomas (also know as von Meyenburg complexes) and bile duct adenomas are usually small, incidental asymptomatic lesions identified at time of autopsy. Bile duct hamartomas are believed to be formed by failure of the embryonic ductal plates in the liver to involute. These lesions are often subcapsular, small white nodules that may require differentiation from metastatic adenocarcinoma or cholangiocarcinoma (211). Bile duct adenomas are also small subcapsular nodules consisting of acini and tubules and may be confused for a malignant lesion (212). Both of these benign bile duct proliferations have an immunohistochemical profile similar to that of pancreatic ductal adenocarcinoma and are positive for CK7, CK17, MUC1 and MUC5AC (213). They also share an immunophenotype with bile duct carcinoma, and are all positive for CK7, focally positive for CK20, and CDX-2; however, they are negative for p53 and monoclonal CEA which is positive in bile duct carcinoma (214). Hence, it is important to correlate with radiological and clinical findings.

**Conclusions**

Tumors of the gastrointestinal tract are varied, yet can often prove to be diagnostically challenging. Understanding the unique immunohistochemical profiles of each entity will greatly assist in the diagnosis of these tumors. Table 3 provides a summary of the immunohistochemical profile of several key gastrointestinal tumors.

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