Pathological Evaluation, Classification, and Staging of Colorectal Cancers

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Pathological Evaluation

Sample Processing

It is important to be aware of the path that biopsied or resected tissue follows from the time it leaves the operating room or the endoscopy suite until the pathologist renders her diagnosis. Biopsy samples are usually submitted in containers with a chemical fixative such as formalin from the endoscopy suite so if studies that require fresh tissue are desired at the time of endoscopic biopsy, this must be arranged in advance with the endoscopist. However, many modern assays are standardized for use on formalin-fixed paraffin-embedded tissue. All specimens must be grossly (macroscopically) evaluated prior to processing for microscopic evaluation. Gross examination of a specimen or "grossing" is the inspection of the specimen with the naked eye to obtain diagnostic information and document precisely what was biopsied or resected from the patient. The first step in gross examination of a specimen is to confirm patient identity and the exact anatomical location from which the specimen was obtained. Whereas it is simple to identify the anatomic site of resection specimen that contains a segment of ileum, an appendix, and a length of colon, it is impossible to separate anatomic sites by gross evaluation of mucosal pinch biopsies, such that careful attention to labeling by the endoscopist is critical. The gross appearance of the specimen is documented and is included in the final pathology report.

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Biopsy samples are typically small and submitted for processing "whole," whereas tissue from resection samples is cut with a razor blade into postage stamp-sized portions that fit in plastic cassettes that are processed to make paraffin tissue blocks. These tissue blocks are sectioned into 5- to 10-micron-thin sections positioned on glass slides for staining and microscopic examination. The histological sections are stained with the hematoxylin and eosin (H&E) stain for evaluation by a pathologist. In the vast majority of cases, an accurate interpretation can be made by the use of H&E stains alone.

Gross Evaluation of Small Biopsies and Large Polyps

Small colonic biopsies are minute fragments of tissue taken by pinch biopsy forceps. They typically measure from 0.5 mm to 3–4 mm, depending on the type of forceps used and the endoscopist. Gross evaluation of small biopsies includes recording the number of the fragments received and measuring the aggregate dimension and, in some instances, measuring the size of the largest fragment. Ideally, there should be no more than three fragments of tissue submitted per container. As the number of the tissue fragments increases on a slide, the possibility of unwanted error increases. Obviously, documenting the exact site from which the biopsy was obtained and documenting the type of tissue (such as polyp or mass versus flat mucosa) is essential for the pathologist to render the correct diagnosis.

Polyps removed during colonoscopy can be small or large, pedunculated or sessile. The cautery (diathermy) artifact identifies the resection margin in polyps removed with cauterized wire or hot polypectomy. Applying India ink to the stalk also can help the pathologist in identifying the margin of resection at the time of microscopic evaluation, as the applied India ink survives tissue processing and appears black on the glass slides. During pathological gross evaluation, the size, color, surface configuration, and appearance of

S. Yalcin, P. Philip (eds.), *Textbook of Gastrointestinal Oncology*, https://doi.org/10.1007/978-3-030-18890-0_3

any base of a polyp and any visible stalk length should be documented. If the polyp is small (less than 1 cm), it is usually bisected and submitted in 1 cassette. Larger polyps are submitted in more cassettes. The stalk is first inked and then carefully cut and placed in different cassettes in order to evaluate for invasive carcinoma.

Gross Evaluation of a Resected Tumor

Resection specimen processing, not surprisingly, is more complicated than processing pinch biopsies. The first step in the gross examination after patient identification is to record the exact anatomical subdivision of the resected colon. We cannot emphasize enough the importance of correctly labeling the anatomical portion of the colon (e.g., sigmoid versus rectum versus rectosigmoid). Furthermore, it is impossible to distinguish the subdivisions of the colon, such as ascending versus transverse versus descending colon, by gross examination. Anatomically, serosa and taenia coli are present from the right colon until the sigmoid and are absent in the posterior rectum and anus. There is a serosal covering in the anterior upper rectum and the upper rectal sides. Mesentery, on the other hand, is absent in cecum and rectum and present in the transverse and sigmoid colon.

It is crucial for the pathologist to perform a careful gross examination of the external surface of the specimen and document any extension of tumor to the outer surface. Measuring the length and circumference of the specimen should be done before opening. The specimen should be received intact from the operating room. On rare occasions, some surgeons choose to open the colon in the operating suite. We strongly advise against it, as it may hamper pathological evaluation of the specimen including assessment of the depth of invasion (T stage) and the distance of the tumor from the margins (Table 3.1). For the concerned surgeon, issues of completeness of resection can usually be answered by the pathologist in the form of an intraoperative consultation.

Identifying the radial circumferential margin is a crucial step in grossing the rectal lesions. The rectosigmoid junction is where the peritoneum no longer completely surrounds the large bowel. The rectum is partially covered by peritoneum in the upper third (on the anterior and lateral sides) and in the middle third (only the anterior aspect). No peritoneum covers the lower third of the rectum. The exact location of the tumor must be identified and recorded. Thus, correctly labeling the specimen as rectal prompts the pathologist to identify the peritonealized versus nonperitonealized zones. Careful pathological grossing evaluation through orientation of the

Table 3.1 TNM staging of colorectal cancers

Definition of primary tumor (T)	
T category	T criteria
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ*:* intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
T1	Tumor invades the submucosa (through the muscularis mucosa but not into the musclaris propria)
T2	Tumor invades muscularis propria
T3	Tumor invades through the muscularis propria into pericolorectal tissues
T4	Tumor invades the visceral peritoneum or invades or adheres to adjacent organ or structure
T4a	Tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
T4b	Tumor directly invades or adheres to adjacent organs or structures
Definition of regional lymph node (N)	
N category	N criteria
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	One to three regional lymph nodes are positive (tumor in lymph nodes measuring ≥0.2 mm), or any number of tumor deposits are present and all identifiable lymph nodes are negative
N1a	One regional lymph node is positive
N1b	Two or three regional lymph nodes are positive
N1c	No regional lymph nodes are positive, but there are tumor deposits in the Subserosa Mesentery Nonperitonealized pericolic, or perirectal/mesorectal tissues
N2	Four or more regional nodes are positive
N2a	Four to six regional lymph nodes are positive
N2b	Seven or more regional lymph nodes are positive
Definition of distant metastasis (M)	
M category	M criteria
M0	No distant metastasis by imaging, etc.; no evidence of tumor in distant sites or organs (this category is not assigned by pathologists.)
M1	Metastasis to one or more distant sites or organs or peritoneal metastasis is identified
M1a	Metastasis to one site or organ is identified without peritoneal metastasis
M1b	Metastasis to two or more sites or organ is identified without peritoneal metastasis
M1c	Metastasis to the peritoneal surface is identified alone or with other site or organ metastasis

Table 3.1 (continued)

AJCC prognostic stage groups			
When T is…	And N is…	And M is…	Then the stage group is…
Tis	N0	M0	0
T1, T2	N0	M0	I
T3	N0	M0	IIA
T4a	N0	M0	IIB
T4b	N0	M0	IIC
T1-T2	N1/N1c	M0	IIIA
T1	N2a	M0	IIIA
T3-T4a	N1/N1c	M0	IIIB
T2-T3	N2a	M0	IIIB
T1-T2	N2b	M0	IIIB
T4a	N2a	M0	IIIC
T3-T4a	N2b	M0	IIIC
T4b	N1-N2	M0	IIIC
Any T	Any N	M1a	IVA
Any T	Any N	M1b	IVB
Any T	Any N	M1c	IVC

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specimen, inking, and evaluation of the margins allows assessment of completeness of the excision. The distance of the tumor to the closest margin, especially in the rectum, is an important prognostic factor. Ideally, in order to be able to properly section the specimen it is pinned to a corkboard or a wax board and fixed overnight in formalin. Representative sections of all of the components present (e.g., appendix, cecum, ascending colon) and any visible lesions are submitted for microscopic evaluation. The fat is stripped off of the specimen to identify all the lymph nodes.

Classification of the Colorectal Cancers and Precursor Lesions

Colorectal Polyps

Colonic polyps can be generally categorized as conventional adenomas or serrated polyps. Tubular or tubullovillous adenomas account for approximately 60% of colonic polyps. Serrated polyps are categorized as, hyperplastic polyps (HPs), sessile serrated adenoma (SSA), traditional serrated adenomas (TSAs), and SSA with cytological dysplasia (formerly termed mixed hyperplastic/adenomatous polyps [MHPAPs]). SSA with cytological dysplasia accounts for 1–2% of colonic polyps [1].

Tubular Adenoma

Tubular adenomas are considered precursor lesions to carcinomas. It is believed that about 10% of adenomas that are not removed will transform to adenocarcinomas [2]. Histologically, tubular adenomas are composed of cells with elongated pseudostratified hyperchromatic nuclei (Fig. 3.1). These nuclei maintain their polarity, meaning their long axis is perpendicular to the basement membrane (Fig. 3.2). Adenomas may contain scattered neutrophils, prominent apoptosis, Paneth cell differentiation, clear cell change, and squamous-like morules. Adenomas with so-called pseudoinvasion contain neoplastic glands that, together with their lamina propria, prolapse into the submucosa. These glands can become obstructed and the inspissated mucin can dissect through the tissue mimicking invasive carcinoma. In most

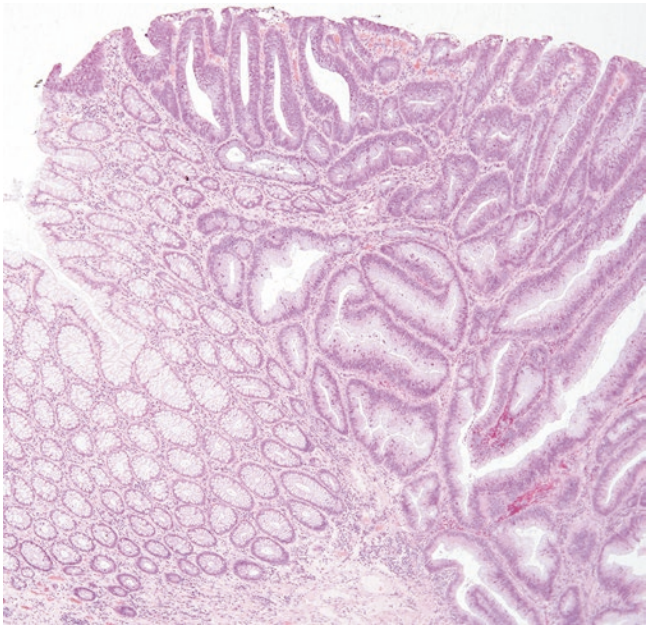

Fig. 3.1 Tubular adenoma. These polyps show hyperchromatic, pencil-like nuclei that remain perpendicular to the basement membrane (on the left) as compared to the normal mucosa (on the right)

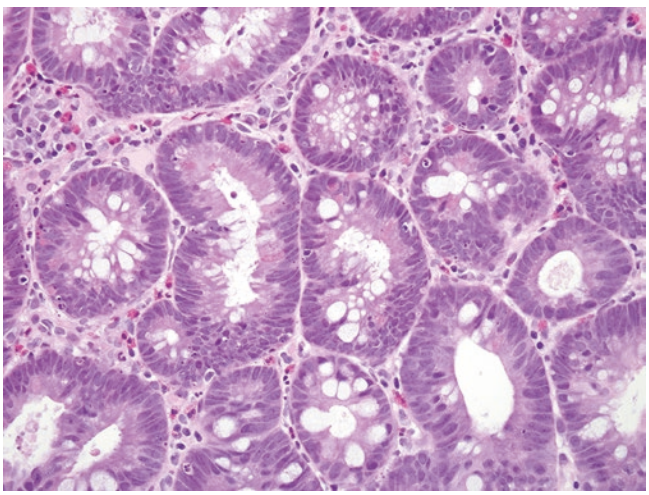

Fig. 3.2 Tubular adenoma. This figure highlights the elongated, hyperchromatic nuclei that are perpendicular to the basement membrane

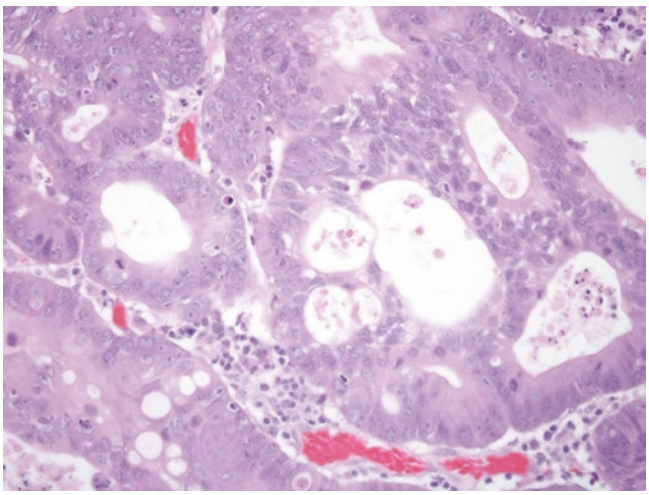

Fig. 3.3 Adenoma with high-grade dysplasia. This high-magnification image shows an area of cribriform architecture with loss of nuclear polarity and bizarre cells

cases, an expert pathologist can differentiate such pseudoinvasion from true invasion based on the presence of lamina propria, hemosiderin, round glands, and cytoarchitectural features.

Adenomas have low-grade dysplasia by definition. The presence of high-grade dysplasia in a tubular adenoma warrants a more frequent follow-up [3]. Cribriform architecture and/or loss of nuclear polarity along with cytological atypia and stratification of the nuclei to the surface or the gland lumina define high-grade dysplasia in an adenoma (Fig. 3.3). Intramucosal carcinoma happens when there is lamina propria invasion. Since the lamina propria of the colon lacks significant lymphatics, this early invasion is staged as Tis rather than T1 (submucosal invasion) (Table 3.1). In both occasions, whether there is high-grade dysplasia or intramucosal carcinoma in an adenoma, polypectomy should be curative. Tubulovillous adenomas show a mixture of tubular and villous architecture and villous adenoma is a polyp displaying predominantly villous architecture. Villous adenomas are believed to warrant closer surveillance than tubular adenomas, but the cutoff between the 2 is poorly defined.

Hyperplastic Polyps

Classical HPs are incidental findings during routine colonoscopy and account for the majority (about 75%) of all serrated polyps. They can be single or multiple, usually less than 5 mm, and commonly found in the rectosigmoid region. Histologically, they can be recognized as microvesicular, goblet cell-rich and mucin-poor variants. However, since these subtypes have no clinical significance, there is no need to subclassify them during routine histological examination.

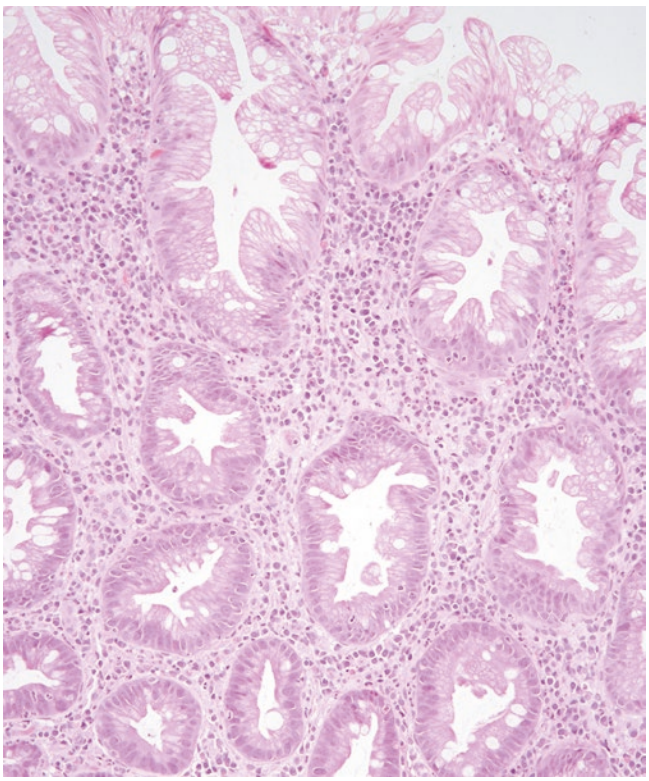

Fig. 3.4 Hyperplastic polyp. This example shows star-like glandular morphology in the upper crypts

Morphologically, HPs show serrated or star-like glandular morphology in the upper crypts with glands tapering down near the base with prominent neuroendocrine cells (Fig. 3.4). Some of these polyps might have a regular thickened collagen table. Microvesicular HPs have frequent BRAF mutation while goblet cell-rich HPs more commonly have KRAS mutation supporting the evidence of identifying and removing these polyps during endoscopy [1].

Sessile Serrated Adenoma/Sessile Serrated Polyp

Sessile serrated adenoma (SSA) and sessile serrated polyps (SSPs) account for 15–25% of all serrated polyps. Endoscopically, they may be subtle and difficult to distinguish from a thickened mucosal fold. They are broad-based and more commonly arise in the right colon and may attain a size of several centimeters. These polyps are characterized by serrated crypt architecture extending to the deep crypts with dilated crypt bases that are aligned parallel to the muscularis mucosae (Fig. 3.5). Morphological variability exists in these polyps. They can have oncocytic changes or increased or decreased mucin that sometimes resembles gastric foveolar epithelium. Conventional-appearing low-grade dysplasia can arise in SSA, characterized by loss of expression of MLH1 and/or PMS2 by immunohistochemistry.

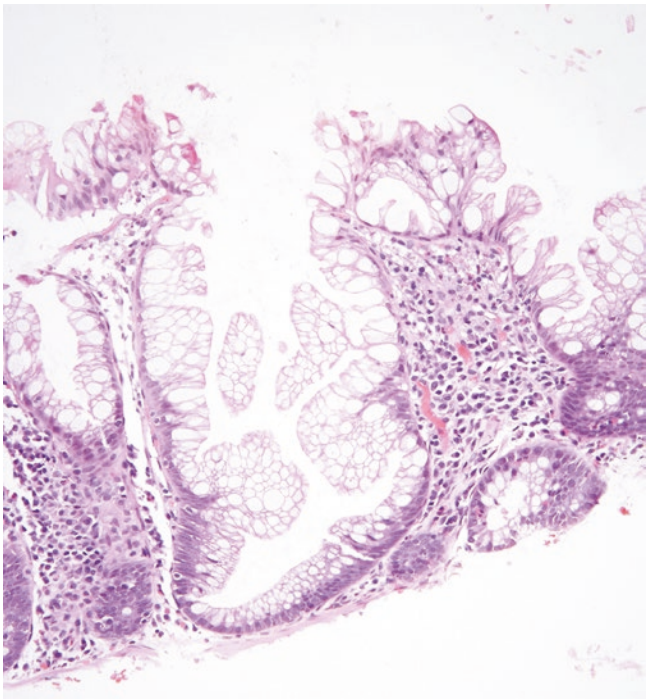

Fig. 3.5 Sessile serrated adenoma. Note the serrated crypt architecture extending to the deep crypts with a dilated crypt base aligned parallel to the muscularis mucosae

SSAs often harbor activating mutation in the *BRAF* gene, interfering with cellular apoptosis and thereby causing epithelial cells to accumulate over basement membrane producing serrated areas. Most SSAs (67%) have aberrant nuclear beta-catenin labeling, seen in the background of *BRAF* mutations and correlating with neoplastic progression [4].

Traditional Serrated Adenoma

Traditional serrated adenomas (TSAs) occur predominately in the distal colon. Histologically, these polyps are characterized by complex villiform architecture with crypts that lose their orientation to the muscularis mucosae and bud off disorganized glands (ectopic crypts). The lesional cells of TSA have brightly eosinophilic cytoplasm and cigar-shaped nuclei that are shorter than those of typical tubular adenoma (Fig. 3.6). These nuclei lack significant enlargement, prominent nucleoli and apoptosis. TSA are characterized by *KRAS* mutations and CpG island methylation, but they lack microsatellite instability (MSI) unlike SSAs that have progressed to dysplasia of carcinoma.

Filiform serrated adenoma is an uncommon variant of TSAs found on the left side of the colon. Complex delicate fronds, abundant eosinophilic cytoplasm and tiny crypts emanating from the surface of the fronds and edematous stroma characterize these polyps (Fig. 3.7). These polyps can be associated with areas of conventional tubular adenoma,

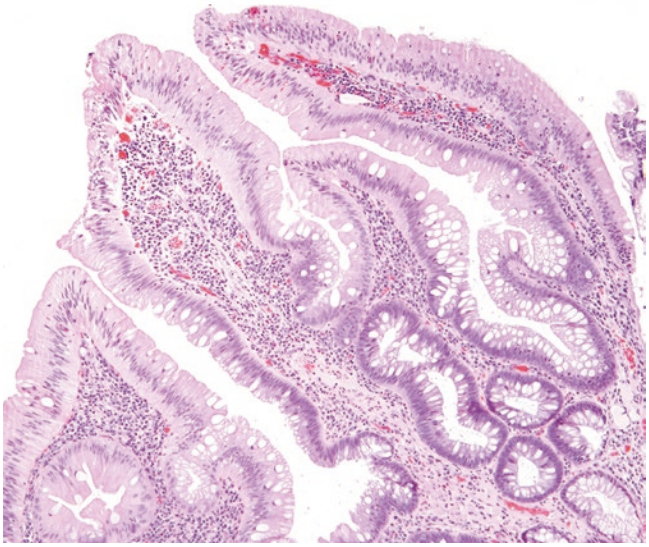

Fig. 3.6 Traditional serrated adenoma. This example shows the pink (eosinophilic) cytoplasm of these adenomas. Note the nuclei are smaller than those of a tubular adenoma

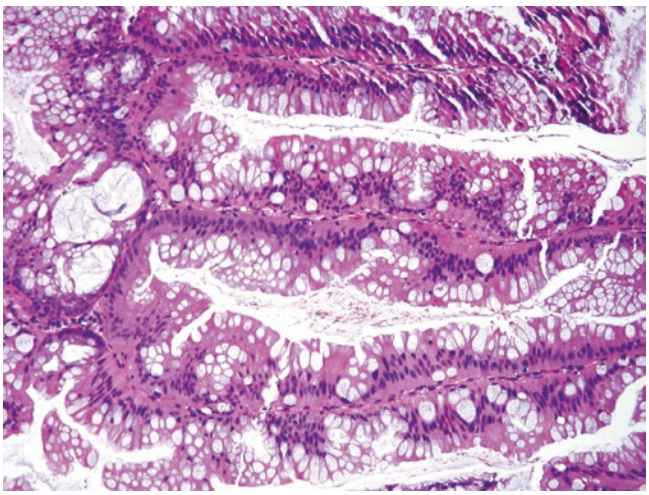

Fig. 3.7 Filiform serrated adenoma. Note the delicate fronds, abundant eosinophilic cytoplasm, and tiny crypts emanating from the surface of the fronds (ectopic crypts)

high-grade dysplasia, SSAs, or HPs. These polyps are molecularly similar to SSAs as they harbor *BRAF* mutation in approximately 50% and a minority with *KRAS* mutation around 21%. Filiform serrated adenomas are microsatellite stable or have low levels of microsatellite instability [5].

Malignant Adenoma (Adenocarcinoma in Adenoma, "Malignant Polyp")

Malignancy in adenomas or adenoma containing invasive carcinoma is defined by invasion of the tumor through the muscularis mucosae into the submucosa (Fig. 3.8). The

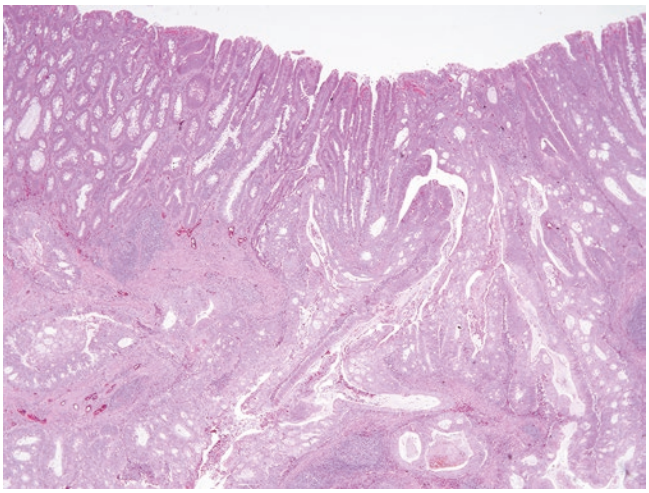

Fig. 3.8 Malignant adenoma (adenocarcinoma arising in association with an adenoma). Note the invasion of the tumor through the muscularis mucosae into the submucosa

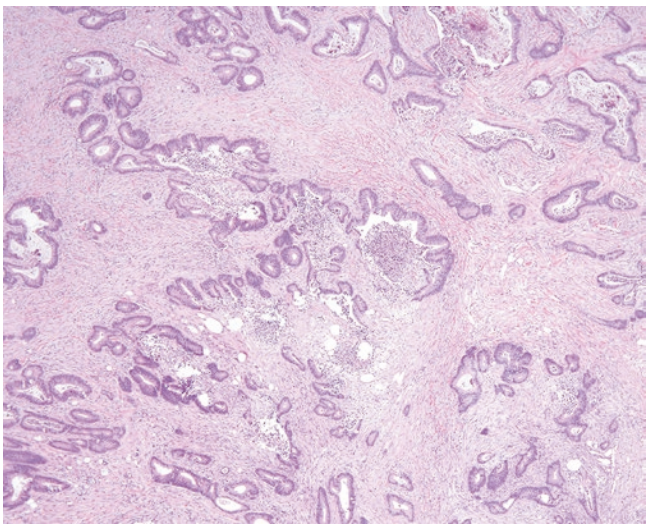

Fig. 3.9 Colonic adenocarcinoma. This example shows the angulated glands with central necrosis in a desmoplastic stroma

chance of finding an invasive carcinoma component in a polyp increases with increasing adenoma size. The likelihood of finding an invasive carcinoma component in an adenoma larger than 2 cm is approximately 35–53%. Ideally, large polyps should be resected intact (if possible) in order for the pathologist to be able to identify the margin of resection and asses the closet approach of tumor.

Many so-called malignant polyps are curable by endoscopic polypectomy alone. Criteria that have been offered to determine which such lesions require follow-up resection to harvest lymph nodes include: (1) high tumor grade, (2) tumor present ≤1 mm (or some references 2 mm) from the resection margin, and (3) small vessel invasion. Higher tumor grade includes poorly differentiated adenocarcinoma, signet ring cell carcinoma, small cell carcinoma or undifferentiated carcinoma and may be similar to so-called tumor budding. In the presence of any of these features, the risk of an adverse outcome is increased to 10–25% [6].

Colitis-Associated Dysplasia

Dysplasia in inflammatory bowel disease (IBD) is classified as low and high grade, and in unclear cases indefinite for dysplasia. The presence of active inflammation with reactive epithelial changes makes the diagnosis of low-grade dysplasia sometimes challenging in these patients. The diagnosis of low-grade dysplasia requires the presence of nuclear alteration extending to the surface epithelium. In contrast, high-grade dysplasia displays surface loss of nuclear polarity. Serrated epithelial changes also can be seen in these patients. A recent study demonstrated high frequency of dysplasia in the patients with serrated epithelial changes [7]. However, more studies are needed to determine if these serrated changes are precancerous.

There are some criteria to differentiate sporadic adenoma from polypoid colitis-associated dysplasia; however, no definite criteria exist for this distinction. Patients with polypoid colitis-associated dysplasia are usually younger (<50 years old) with duration of IBD more than 10 years and have active disease. Endoscopically, the polyps are ill defined versus the sporadic adenomas, which are usually well marginated. Histologically, polypoid-associated dysplasia display irregular gland configuration with a mixture of non-neoplastic and neoplastic glands and variable stroma, irregular mucin production, dystrophic goblet cells, and stratified nuclei in variable levels.

Colorectal Adenocarcinoma

The vast majority of colorectal adenocarcinomas can be diagnosed on a colonic mucosal biopsy with routine H&E staining. Histologically, colorectal adenocarcinomas are characterized by angulated glands and single cells with a desmoplastic stroma. These glands frequently contain necrotic debris, apoptosis, and scattered neutrophils (Fig. 3.9). As previously noted, submucosal invasion is necessary for the diagnosis of invasive adenocarcinoma. For obvious reasons, mucosal biopsies of the colon rarely contain abundant submucosa. However, the presence of well-developed desmoplasia in the lamina propria with associated invasion into the structure is almost invariably accompanied by an underlying invasive carcinoma that extends into at least the submucosa. Sometimes, in fragmented specimens, it is not possible to reach a diagnosis of adenocarcinoma. In cases for which we believe the invasion might be limited to the lamina propria, we report the findings as "at least intramucosal carcinoma/ invasion into the lamina propria/

Tis." Pathologists generally also report any associated adenoma component to note that the lesion is primary rather than a metastasis from another site.

Molecular Testing in Colorectal Carcinoma

Molecular testing can be performed on both biopsies and resection specimens. However, since staging is a factor in determining whether molecular testing is indicated, we often wait until the resection when staging data are available and there is abundant material for testing. Microsatellite instability (MSI) is generally performed for all patients under 70 with stage 1 tumors, all patients with stage 2 tumors, all patients under 70 with stage 3 tumors, and for all stage 4 tumors. Microsatellites or short tandem repeats (STRs) are repetitive DNA elements of 1–6 base units. These units are repeated 10–60 times, which creates inherent instability during replication. These errors are corrected through a system of DNA mismatch repair (MMR) in normal cells. Proteins encoded by the genes in this system, such as mutL homolog 1 (hMLH1), postmeiotic segregation increased 2 (hPMS2), mutS homolog1 (hMSH2), and mutS homolog 6 (hMSH6) genes.

There are some histological findings that are suggestive of MSI in colorectal tumors. These include intense lymphocytic intretumoral infiltrates (Fig. 3.10), mucinous (Fig. 3.11) or signet ring features, and Crohn-like peritumoral features. In fact, noting a combination of these features in a patient younger than 50 can predict MSI with great accuracy [8]. MSI testing involves microdissecting the tumor and the normal tissue from sections prepared from the paraffin-embedded tissue blocks and performing polymerase chain reaction (PCR) using primers directed to microsatellite markers. Therefore, we always encourage our clinical colleagues to also sample the normal mucosa in a young patient of less than 50 years old. However, this testing can be done later on the resected material where normal tissue is readily available. Different systems with different numbers of mononucleotides or dinucleotides marker are available for MMR testing. Several patterns of data interpretation exist: MSI-high (MSI-H), MSI-low (MSI-L), and microsatellite stable (MSS). When using 5 markers, MSI-H corresponds to greater or equal to 2 loci of MSI, when only 1 loci shows MSI is considered MSI-L and MSS is defined by no detected MSI.

Immunohistochemical staining is an alternative route to MMR testing. Pathologists can stain the tissue with surrogate markers for the presence of MMR gene mutations and such testing generally correlates well with MSI testing. MLH1, MSH2, MLH6 and PMS2 are the proteins encoded by the MMR genes and loss of immunolabeling will indicate a defective gene or, in the case of MLH1, inactivation of the gene by promotor methylation. Using immunohistochemistry also directs the clinician to order gene sequencing on the defective gene. As mentioned earlier, the lack of immunostaining does not preclude the possibility of inactivation of the gene by promotor methylation or a missense mutation that causes loss of function of the protein.

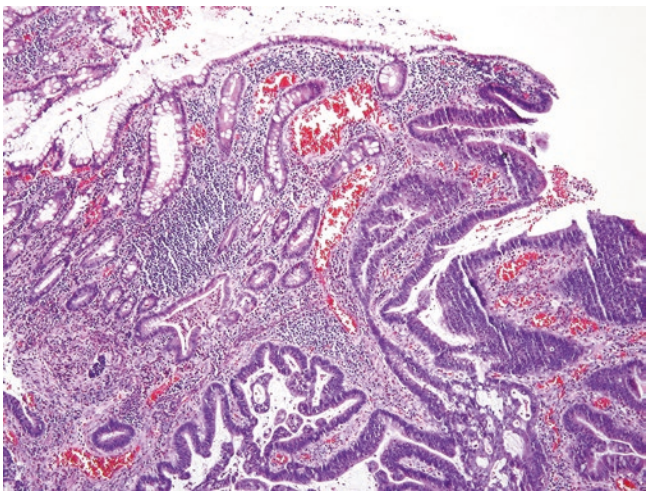

Fig. 3.10 Colon carcinoma associated with microsatellite instability (MSI). Note the presence of an adenocarcinoma with an intense lymphocytic infiltrate

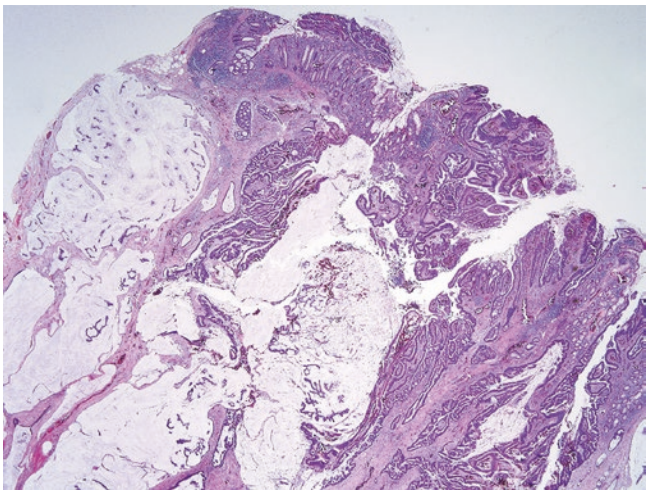

Fig. 3.11 Colon carcinoma associated with microsatellite instability (MSI). This an example of adenocarcinoma with mucinous features. Note the pools of mucin with floating malignant cells

Currently, *BRAF* and *KRAS* mutation status are commonly tested to guide therapeutic options as tumors that harbor these mutations are resistant to anti- epidermal growth factor receptor (EGFR) immunotherapy. Since these drugs are expensive and have significant morbidity, testing the gene is recommended. *KRAS* and *BRAF* mutational testing is indicated in any stage III or IV tumor; that is, any tumor that has spread to lymph nodes or distant sites. Common labora-

tory methods used are gene sequencing and real-time PCR. Many laboratories test the *KRAS* gene first and if it is mutated there is no need to test the *BRAF* gene, as the patient would be expected to be resistant to anti-EGFR therapy.

Hereditary Colorectal Cancer Syndromes

Familial Adenomatous Polyposis

Patients with familial adenomatous polyposis (FAP) have a germline mutation in the adenomatous polyposis coli (*APC*) gene on chromosome 5q21 with complete penetrance, causing them to have hundreds to thousands of colonic adenomas (Fig. 3.12). These patients essentially all develop colon cancer without prophylactic colectomy. The proband usually manifests more than 100 or 1000 colonic adenomatous polyps. In these patients, polyposis is not limited to the colon and can involve the stomach and small bowel as well. Thus, even after colectomy, surveillance endoscopies of the upper tract are advised [9]. Morphologically, these tumors are indistinguishable from sporadic adenomas; and the earliest lesions consist of a single neoplastic crypt. The location of the mutation in *APC* gene affects the clinical phenotype that manifests. Thus, full gene sequencing is the standard diagnostic test. Early identification of individuals or their family members with the *APC* gene mutation allows for careful planning and early medical and surgical intervention prior to development of cancer.

Attenuated FAP is a similar dominantly inherited disease with high penetrance. Patients with this disease have fewer than 100 adenomatous colorectal polyps. The location of the *APC* gene mutation in these individuals is at the proximal or distal regions of the gene. Another *APC* gene mutation, I1307K mutation is a missense mutation most commonly found in Ashkenazi Jewish patients. This mutation is also dominantly inherited and increases the risk of developing colorectal cancer up to two- to fivefolds.

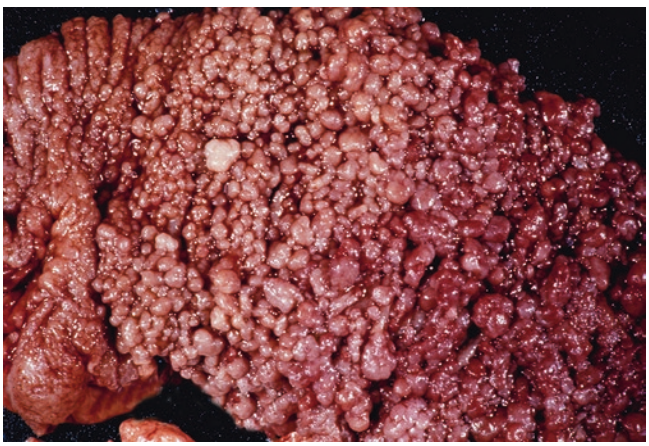

Fig. 3.12 Familial adenomatous polyposis. These patients may have hundreds to thousands of colonic adenomas

MutY-Associated Polyposis

Patients with MutY-associated polyposis have a phenotype similar to that of attenuated APC with less than 100 adenomatous polyps. However, this genetically distinct syndrome demonstrates an autosomal recessive mode of inheritance and is caused by mutation in the *hMYH* gene on chromosome 1.

Hereditary Nonpolyposis Colon Cancer/Lynch Syndrome

Hereditary nonpolyposis colon cancer (HNPCC)/Lynch syndrome accounts for 2–5% of all colorectal cancers and has an autosomal dominant mode of transmission with approximately 80–90% penetrance. The mutated genes in this syndrome are *MLH1* and *MSH2* followed by far fewer examples of mutations in *MSH6* and *PMS2*. Identification of an MMR gene mutation has significant impact on the entire family, leading to close screening and surveillance in those family members carrying the mutation. If a germline MMR mutation arises in a proband, the patient has Lynch syndrome. However, a minority of the colorectal cancers that are mismatch repair deficient are a result of Lynd syndrome. MSI is observed in about 15% of sporadic colorectal cancers mostly occurring in older individuals. Sporadic MSI-H is often due to promotor methylation of *MLH1*, silencing the MMR gene and resulting in loss of expression by immunohistochemistry and the presence of MSI. Thus, genetic testing to differentiate germline origin from sporadic origin is important to confirm Lynch syndrome. *BRAF* testing and MMR gene promotor methylation analysis also can be performed to differentiate sporadic versus germline mutation. MSI-H tumors harboring the *BRAF* V600E mutation are essentially always sporadic.

Serrated Polyposis Syndrome

The World Health Organization (WHO) defining criteria for diagnosing a serrated polyposis syndrome include: (1) the presence of 20 or more serrated polyps throughout the colon, or (2) at least 5 serrated polyps proximal to the sigmoid colon with 2 measuring more than 10 mm, or (3) any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis.

Juvenile Polyposis Syndrome

Juvenile polyposis is characterized by more than 5 juvenile polyps in the colorectal region or the presence of multiple juvenile polyps throughout the gastrointestinal (GI) tract, or any number of these polyps in a patient with a family history of juvenile polyposis. These patients are at risk for colorectal, gastric, duodenal, and pancreatobilliary carcinomas. Juvenile-type polyps can be a component of several genetic syndromes such as juvenile polyposis, Cowden syndrome, and Bannayan-Riley-Ruvalcaba syndrome. Germline mutation in *DPC4* (also known as *SMAD4*) and *BMPR1A* predispose an individual to juvenile polyposis. Mutations in *PTEN,* a tumor suppressor gene, have been documented in Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome. Although these syndromes have different mutations they share similar juvenile-type polyps. A combined syndrome of juvenile polyposis and hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease) is described in patients with *SMAD4* mutations [10].

Sporadic juvenile polyps usually arise in children and usually have a spherical lobulated surface that is often eroded (Fig. 3.13). These polyps are considered hamartomatous. As such, when they arise in the colon they have colonic mucosa with irregularly shaped and dilated glands accompanied by lamina propria that is expanded by granulation tissue (Fig. 3.14). In syndromic patients, smaller polyps have the same features as sporadic juvenile polyps. However, the larger polyps display a relative increase in the epithelium compared to stroma with multilobulation or fingerlike lobes. These syndromic polyps can harbor true dysplasia (intraepithelial neoplasia) that can progress to carcinoma (Fig. 3.15). These polyps can show areas of erosion and active inflammation. Pathologists and clinicians should consider the possibility of these syndromic diseases when encountering nonspecific inflammatory polyps without associated prior mucosal injury. Of note, adult patients can develop inflammatory polyps that are presumably a result of prior mucosal injury and that can mimic juvenile polyps. Pathologists tend to report these cases as inflammatory/juvenile-type polyp.

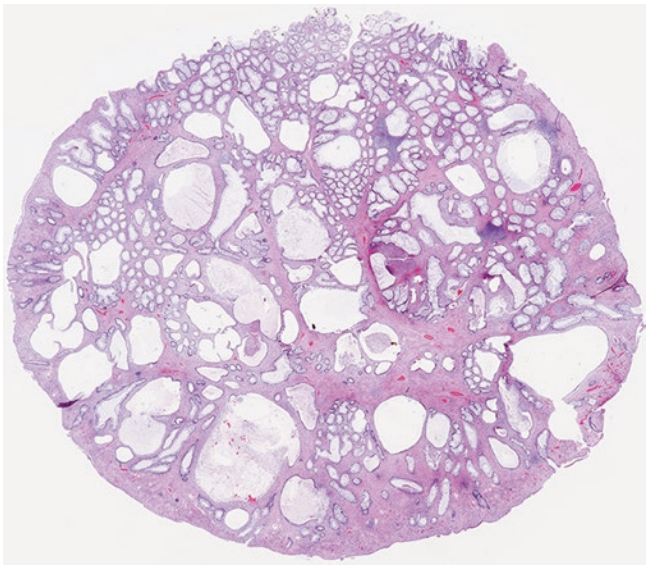

Fig. 3.14 Juvenile polyp. Microscopically, these polyps consist of numerous cystic and dilated glands with edematous stroma and associated lymphocytes and plasma cells

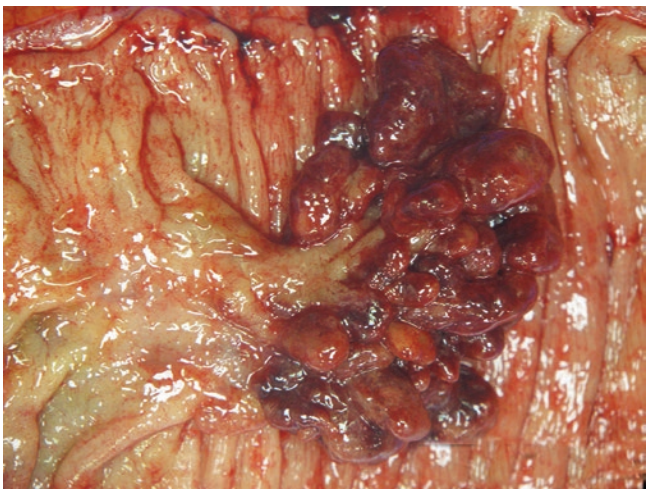

Fig. 3.13 Juvenile polyp. Gross examination of the resected colon shows a lobulated and pedunculated juvenile polyp

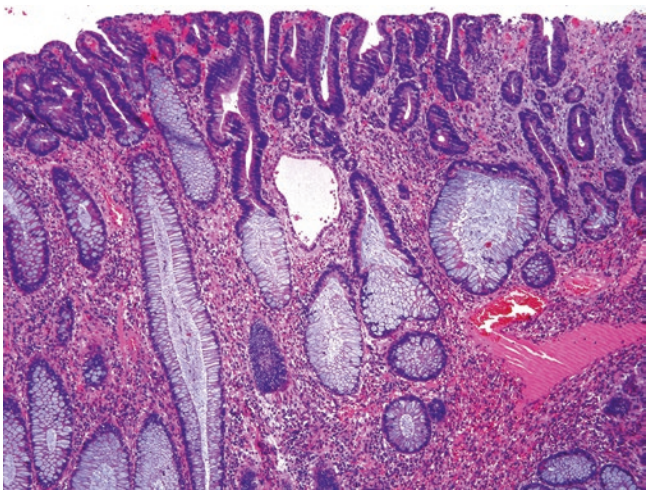

Fig. 3.15 Juvenile polyp with low-grade dysplasia. This example shows a juvenile polyp with low-grade dysplasia showing elongated pseudostratified hyperchromatic nuclei in the surface

Peutz-Jegher Syndrome

Peutz-Jegher (PJ) syndrome is an inherited cancer syndrome characterized by intestinal polyps and mucocutaneous melanin pigmentation. The most common malignancy in this syndrome is colorectal cancer followed by breast, small bowel, stomach, and pancreas carcinomas. Other extraintestinal malignancies arise in the endometrium, lung, ovary (sex cord tumors with annular tubules), cervix (adenoma malignum), and testis (Sertoli cell tumors). The average age at diagnosis of malignancies in patients with PJ syndrome is 42 years [11]. This syndrome is an autosomal dominant one with virtually 100% penetrance and is associated with mutation in the *LKB/STK11* gene in 80–94% of cases [12, 13]. The polyps in PJ syndrome are most common in the small intestine but can also arise in the colon and stomach.

Histologically, PJ polyps are hamartomatous and display the type of mucosa typical for the site in which they are found. Thus, in the stomach, they have gastric mucosa and in the colon they have colonic mucosa. These polyps characteristically display arborizing smooth muscle cores from which the mucosa leafs out (Fig. 3.16). The problem in diagnosing these polyps on mucosal biopsies arises when biopsies are superficial or when ulceration distorts the architecture. In the colorectal region, mucosal prolapse is very common and characterized by the presence of smooth muscle in the lamina propria and thus, it is difficult to prospectively diagnose PJ syndrome based on a colonic polyp in isolation. This diagnosis should be made in the context of clinical history. Although these polyps can have dysplasia or associated invasive carcinoma [14], most GI malignancies do not arise from the polyps themselves.

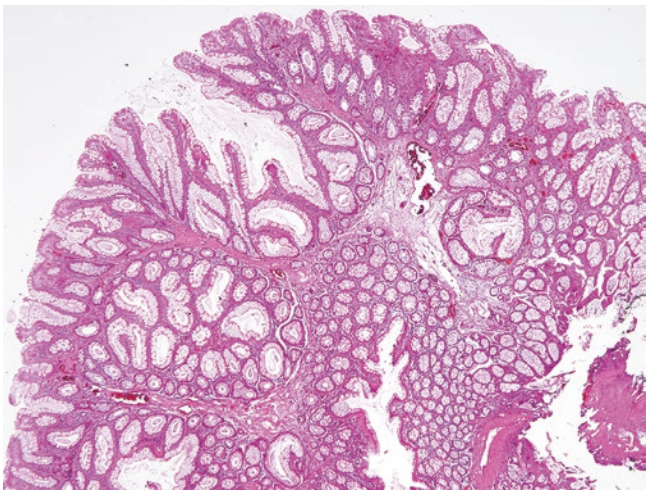

Fig. 3.16 Peutz-Jegher polyp. Note the arborizing smooth muscle separating groups of glands

Cronkhite-Canada Polyps

Cronkhite and Canada first reported this syndrome in 1955 in a series of patients with polyposis, pigmentation, alopecia, and onychotrophia [15]. Several studies afterward were able to further characterize this syndrome; however, the polyps arising in Cronkhite-Canada syndrome are impossible to prospectively diagnose based on microscopic features in isolation [16]. Cronkhite-Canada syndrome is characterized by diffuse polyposis in patients with unusual ectodermal abnormalities, including alopecia, onychodystrophy, and skin hyperpigmentation. The mean age of onset is around 59 years with a male-to-female ratio of 3:2, and it has been reported mostly in Southeast Asians and Europeans. This syndrome can have fatal complications such as malnutrition, GI hemorrhage, and infection, with a mortality rate as high as 60%. The most common presenting symptoms include diarrhea, weight loss, hypogeusia, and anorexia. Paraesthesias, seizures, and tetany have also been recorded. The poor outcome of these patients reflects a number of complications such as fatal GI bleeding, intussusception, prolapse, and malabsorption leading to malnutrition and recurrent infections.

Cronkhite-Canada syndrome is distinguished by the diffuse distribution of the polyps throughout the gastrointestinal tract sparing only the esophagus. It remains controversial whether these polyps have malignant potential. Histologically, broad sessile bases, expanded edematous lamina propria and cystic glands characterize these polyps. These features also can be seen in juvenile polyposis. Additionally, the polyps of Cronkhite-Canada have a pedunculated growth pattern except in the stomach. Clinical correlation with the ectodermal findings is helpful in diagnosing these polyps. Furthermore, if the endoscopist biopsies the flat mucosa in between polyps, it is normal in juvenile polyposis syndrome whereas it is abnormal in Cronkhite-Canada syndrome. The presence of dysplasia favors juvenile polyposis as essentially all Cronkhite-Canada polyps are nondysplastic.

Neuroendocrine Tumors

Most well-differentiated neuroendocrine tumors occur in the GI tract. In the large bowel, they are essentially limited to the rectum [17]. Rectal neuroendocrine tumors are more common in African Americans and Asians and slightly more common in males than females. A small percentage of GI tract neuroendocrine tumors are reported in the ascending colon, most commonly in the cecum. In contrast to the rectal neuroendocrine tumors, the tumors reported in the right colon are nonlocalized in 55–67% and most patients (85%) have metastatic disease at the time of presentation [17, 18].

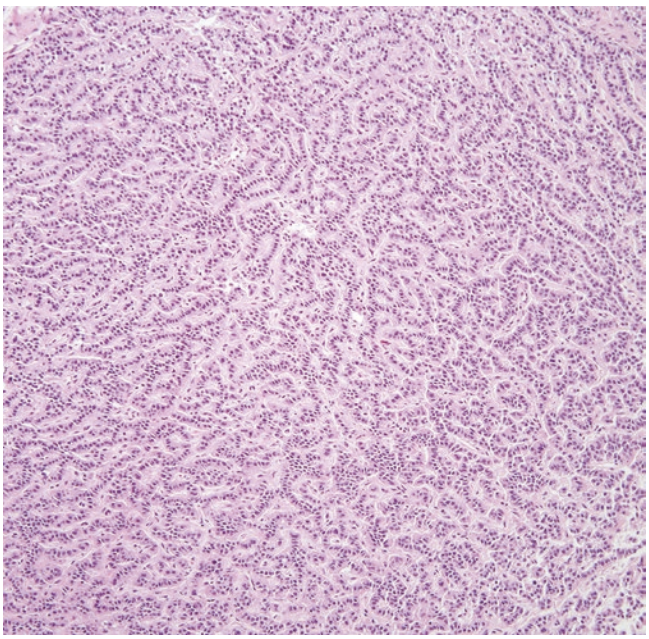

Fig. 3.17 Well-differentiated neuroendocrine tumor. This example has a trabecular growth pattern

Neuroendocrine tumors are classified as well-differentiated and poorly differentiated tumors. Well-differentiated tumors are further divided into G1 or carcinoid tumors and G2 or intermediate. Poorly differentiated neuroendocrine tumors are characterized as G3 (large and small cell type). Histologically, grade 1 and grade 2 tumors are composed of uniform cells arranged in an insular, trabecular, solid, or cribriform pattern (Fig. 3.17). The cells have moderate amount of cytoplasm with round, regular nuclei with a so-called salt-and-pepper chromatin pattern. These lesional cells can frequently form rosettes. The WHO grading system is used for neuroendocrine tumors in the stomach, duodenum, pancreas, and hindgut (colorectal region). This grading system is based on number of mitosis per 10 high power field (HPF) or percentage of MIB1/Ki-67 immunolabeling in lesional cells. G1 is defined based on less than 2 mitoses per 10 HPF or less than 2% Ki67 index. G2 is for mitotic counts between 2 and 20 per 10 HPF or a Ki-67 immunolabeling of 3–20%. Grade 3 tumors are high-grade neuroendocrine carcinomas with small or large cell histology and are characterized by more than 20 mitosis per 10 HPF or more than 20% ki-67 index.

Most well-differentiated neuroendocrine tumors of the rectum are small and localized at the time of presentation and detected at the time of screening colonoscopy. These tumors behave in an indolent fashion and the associated 5-year survival is 90%. Tumor size and invasion of muscularis propria are the two most important predictors of malignant behavior. Small tumors, 1 cm to 2 cm without muscularis propria invasion can be managed by polypectomy. However, even small tumors (between 1 cm and 2 cm) that invade the muscularis propria require transanal excision [17, 19]. Neuroendocrine tumors larger than 2 cm or with regional lymph node involvement are surgically managed as per rectal adenocarcinoma.

Neuroendocrine carcinomas are associated with extensive necrosis, apoptosis, and lymphovascular invasion. The small cell type demonstrates a diffuse growth pattern with round nuclei and nuclear molding. Large cell neuroendocrine tumors usually show a nested pattern of growth with round to oval cells with moderate amount of cytoplasm, granular or vesicular chromatin pattern and visible nucleoli. These tumors can have focal lumen formation and in some instances intracytoplasmic mucin. Of note, high-grade neuroendocrine carcinomas are frequently associated with an adenoma or conventional adenocarcinoma component. Neuroendocrine carcinomas (G3) are aggressive tumors with poor prognosis; however, there is no significant difference between small cell and large cell subtype [20]. Neuroendocrine tumors usually express keratin, synaptophysin, and chromogranin by immunohistochemistry.

Colorectal Sarcomas

Sarcomas of the colon and rectum are very rare and comprise less than 0.1% of all the cancers in this region [21]. Leiomyosarcoma is the most common type of colorectal sarcomas and account for more than 95% [22]. Other sarcomas encountered in this region include Kaposi sarcoma [23], fibrosarcoma [24, 25], angiosarcoma [25], and lipoleiomyosarcomas [26].

Leiomyosarcoma

Most of the colorectal leiomyosarcomas occur in men in the fifth and sixth decade with a predilection for black patients [27]. However, there are case reports of leiomyosarcoma in infants [28]. Histologically, these tumors have perfectly perpendicular fascicles of spindle cells with pleomorphic blunt-ended nuclei with increase abnormal frequent mitosis (Figs. 3.18 and 3.19). Pathologists must differentiate them from gastrointestinal stromal tumors (GISTs), which is easily done by performing immunohistochemistry. Leiomyosarcomas express desmin and not CD117 in contrast to GIST. They also have a better outcome than rectal GIST [29]. Surgical resection is the mainstay of treatment [27].

Kaposi Sarcoma

Kaposi sarcoma (KS) is a quasi-neoplastic sarcoma-like lesion usually encountered in patients with human

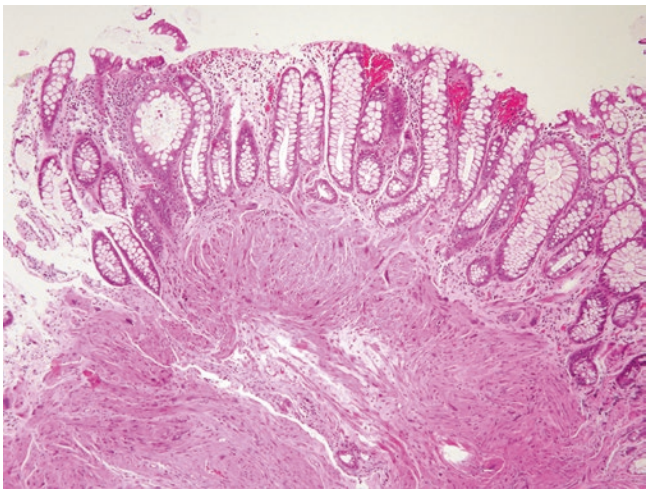

Fig. 3.18 Leiomyosarcoma. This example is extending into the lamina propria and consists of fascicles of spindle cells

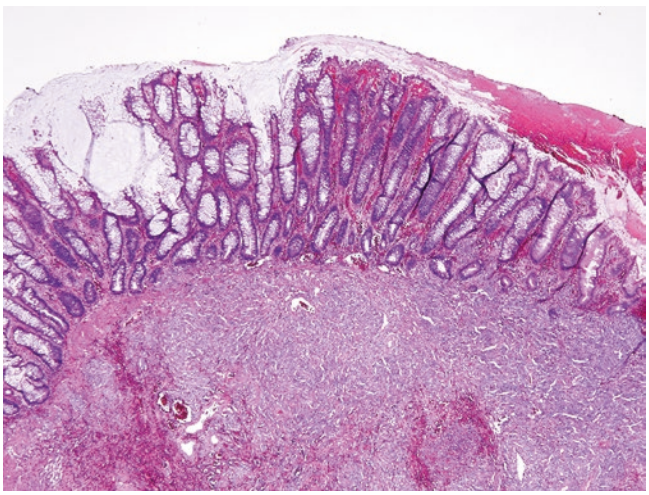

Fig. 3.20 Kaposi sarcoma. This tumor is composed of relatively monomorphic spindled cells, with slit-like vascular channels containing red blood cells

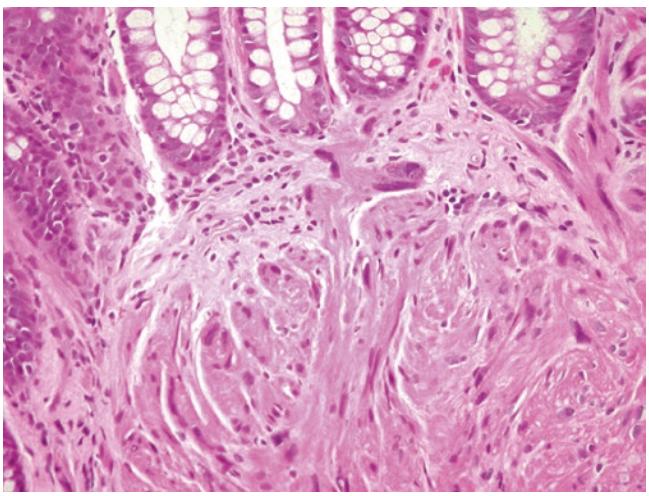

Fig. 3.19 Leiomyosarcoma. This high-magnification image shows the pleomorphic blunt-ended nuclei of a leiomyosarcoma

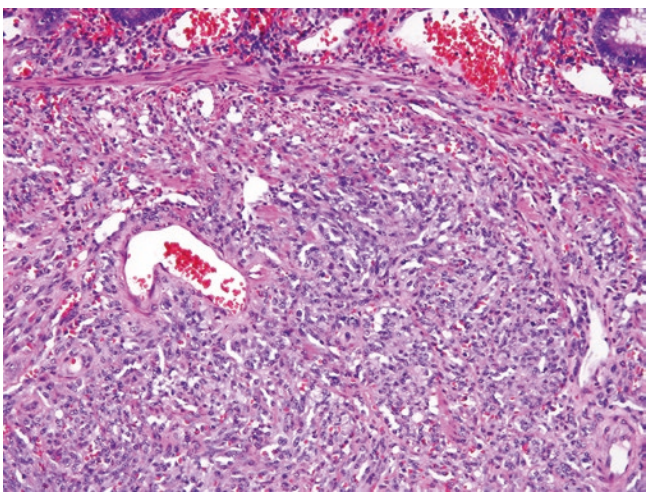

Fig. 3.21 Kaposi sarcoma. This is a higher magnification image of a Kaposi sarcoma highlighting the spindle cells with slit-like vascular channels and erythrocytes

immunodeficiency virus (HIV). KS often involves the skin and lymph nodes, but also can occur throughout the GI tract, including in the rectum and anus. Approximately 40–50% of the HIV patients with cutaneous KS lesions have concurrent lesions in their GI tracts [23]. Rectal KS most often occurs in men who have sex with men (MSM) with HIV with the average age of 34 years [30]. Patients with KS of the GI tract are usually asymptomatic but can have bleeding, diarrhea, or proctalgia. Microscopically, KS is composed of bland spindle cells with prominent red blood cell extravasation (Figs. 3.20 and 3.21). On immunolabeling, the spindle cells express CD34 and CD31 and the diagnosis can be confirmed by demonstration of expression of HHV8 using LAN-1 immunolabeling. Radiation remains the treatment of choice in these patients [21].

Gastrointestinal Stromal Tumors of the Colon

The most common location for GIST in the GI system is the stomach; however, in the lower GI tract region, it often arises in the rectosigmoid colon. They can present with abdominal pain or mass effect. They are usually transmural tumors with intraluminal or outward bulging. Rarely, they can present as subserosal lesions. Histologically, most GISTs are composed of spindle cells in fascicles, palisading with a storiform arrangement or an organoid pattern (Fig. 3.22). Some GISTs can have epithelioid cells as well. By immunohistochemistry, most colonic GISTs are CD117, DOG1, and CD34 positive. Risk assessment of GIST is based on the site, size, and mitotic activity. These tumors are not routinely encountered

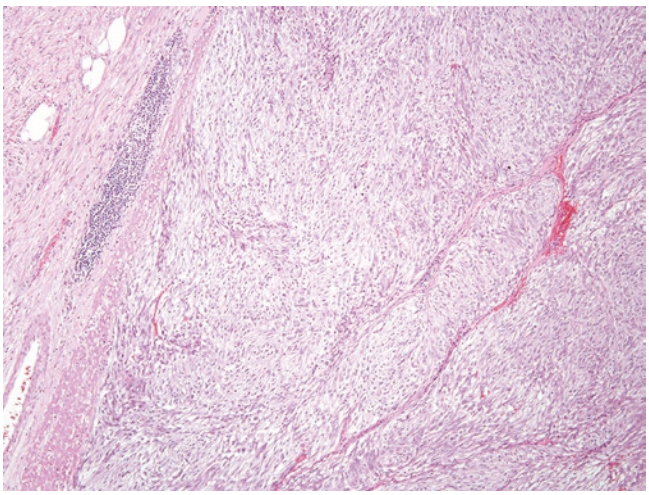

Fig. 3.22 Gastrointestinal stromal tumor (GIST). The tumor consists of monotonous spindle cells arranged in fascicles

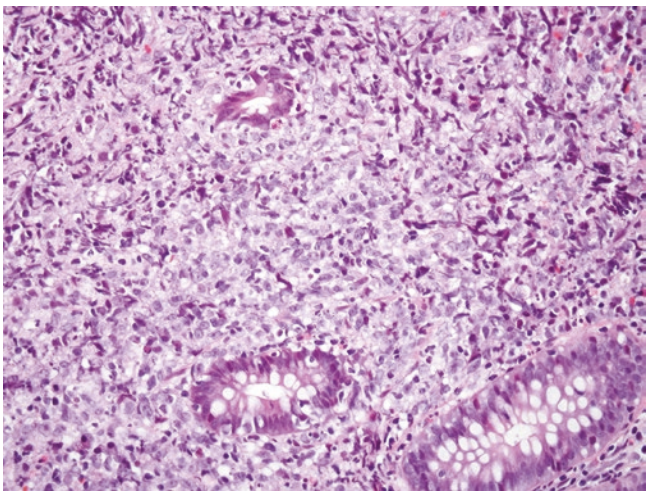

Fig. 3.23 Diffuse large B-cell lymphoma. This example shows the large neoplastic lymphoid cells in the colonic mucosa with apoptosis and inflammatory infiltrate

on colonic biopsies, as they are often transmural. Tumors that invade the mucosa have a worse prognosis.

Lymphoma

Lymphomas are more commonly encountered in the small intestine than the colon or rectum. In the colon, the 2 most common sites of involvement are the cecum and rectosigmoid [31]. Leukemias can also involve the right colon and present with an ischemic colitis pattern.

Diffuse Large B-Cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma subtype affecting the colon. Patients who are immunosuppressed due to HIV, inflammatory bowel disease, and transplantation are at higher risk for lymphomas. Patients with colonic lymphomas can present with abdominal pain, anorexia, weight loss, obstruction, a palpable mass, perforation, or hematochezia [31–33]. Endoscopically, lymphomas manifest as fungating tumors, infiltrative processes, or as ulcerative lesions. Histologically, DLBCL is composed of large cells up to 5 times the size of a normal lymphocyte, with apoptosis and an inflammatory infiltrate (Fig. 3.23). The neoplastic cells can express B-cell markers by immunohistochemistry such as CD19, CD20, CD22, and CD79a. They can also have variable expression of the following antigens: CD10, BCL6, and MUM1.

Follicular Lymphoma

Follicular lymphoma can involve the ileocecal and ascending colon. It can present as multiple mucosal polyps up to 1 cm

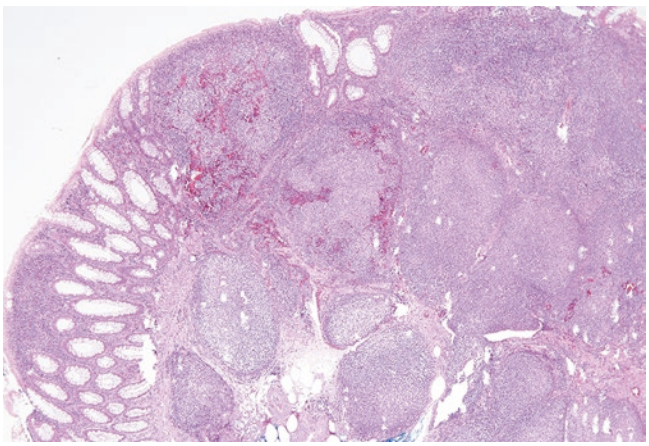

Fig. 3.24 Follicular lymphoma. This example shows the colonic mucosa with exaggerated lymphoid follicles and monotonous germinal centers

[34, 35]. Morphologically, follicular lymphoma presents with exaggerated lymphoid follicles with monotonous germinal centers without typical tingible body macrophages (Fig. 3.24). Most cases are low grade with low mitotic activity. The lesional cells coexpress CD20, CD10, and BCL6 and aberrantly express BCL2 by immunolabeling.

Extranodal Marginal Zone Lymphoma

Mucosal-associated lymphoid tissue (MALT) lymphoma or extranodal marginal zone lymphoma can present as multiple mucosal polyps like mantle cell and follicular lymphoma [35, 36]. Patients can be asymptomatic or present with abdominal discomfort [36]. Histologically, this type of lymphoma is composed of small-to-intermediate size lymphocytes with indented nuclei and abundant cytoplasm involving

the mucosa and submucosa. Immunohistochemically these cells coexpress CD20 and CD43 in 50% of the cases and are negative for CD5, CD10, and CyclinD1. MALT lymphoma has a favorable prognosis and a long-term disease-free survival.

Burkitt Lymphoma

Burkitt lymphoma (BL) is an aggressive B-cell lymphoma that can involve the GI tract, most commonly the ileocecal region and less often the stomach and rectum. BL occurs in 3 clinical forms: (1) endemic, (2) sporadic, and (3) immunodeficiency associated. All 3 forms can present as a bulky mass-forming lesion in the GI tract. Lymph nodes are usually not involved but encased with tumor. Touch imprints and smears can be helpful in diagnosis as the imprint is distinct. The cells on the cytology preparation have deeply blue cytoplasm with lipid vacuoles. Morphologically, the classical and endemic BL is characterized by a "starry sky" appearance composed of sheets of medium size cells (the sky) and scattered tangible body macrophages (the stars). The cells may show squared-off borders with round nuclei and multiple basophilic nucleoli. The atypical pattern has more pleomorphism and fewer nucleoli compared to the classical type. These tumor cells express CD20 and CD10 and lack CD5, BCL2, and TdT. K-i67 immunolabeling shows a very high proliferative index with nearly 100% of the cells being positive.

T-Cell Lymphoma

T-cell lymphoma of the GI tract is rare, principally affecting the small intestine in the setting of gluten sensitive enteropathy [37]. Some cases of primary colonic T-cell lymphoma of the colon have been reported in the Japanese literature in patients with ulcerative colitis [38, 39]. Rare Western cases reported have been associated with gluten sensitive enteropathy [37, 39]. Colonic T-cell lymphoma may present as multiple polyps or multiple shallow or deep ulcers with or without luminal narrowing. Tumor cells are composed of medium-to-large cells with significant cellular pleomorphism, irregular nuclei with small nucleoli and scant-to-moderate amounts of cytoplasm. The tumor cells usually are CD3+, CD4−, CD7+, CD8−, and CD56− and express cytotoxic granule-associated protein TIA-1 often with granzyme B.

Intravascular Lymphoma

Intravascular lymphoma (IVL) or angiotrophic lymphoma is a non-Hodgkin lymphoma that proliferates within the small- and medium-sized blood vessels. IVL usually involves the skin and central nervous system but rarely can affect the lymph nodes, bone marrow, and colorectal region. IVL patients with GI tract involvement present with abdominal pain as a result of bowel ischemia. Colonic biopsies from such patients display ischemic necrosis of the bowel wall with associated vessels containing neoplastic lymphoid cells [40].

Primary Effusion Lymphoma

Primary effusion lymphoma (PEL) is an HHV8-driven lymphoma that usually involves the body cavities in HIV-positive patients. Patients can present with pleural effusion, ascites, and pericardial effusion. In HIV-positive patients, it can accompany Kaposi sarcoma. This neoplasm can rarely present as a solid mass and can involve any part of the GI tract. Morphologically, it is composed of large anaplastic cells with ovoid to irregular nuclei, open chromatin, prominent nucleoli, and moderate amount of pale blue cytoplasm [41]. All cases are positive for HHV8/LAN-1 by immunohistochemistry. Furthermore, these lesional cells are positive for CD45, CD30, and CD138 (plasma cell marker) and are negative for some B-cell markers (such as CD20−, CD19−) and some T-cell markers (CD3− and CD4−). Most cases associated with HIV are coinfected with Epstein-Barr virus (EBV) that can be demonstrated by Epstein-Barr encoding region (EBER) in situ hybridization. These patients with extracavitary PEL have a poor prognosis.

Hodgkin's Lymphoma

Hodgkin's lymphoma can also be encountered in the GI tract; however, the diagnosis should be made with caution. It has been reported in patients with IBD treated with immunomodulation.

Other Tumors

Tumors outside of the colorectal region can either extend or metastasize to the area. These tumors include prostate and bladder carcinoma, tumors of the lung, breast, ovary, and stomach as well as melanoma (Fig. 3.25), mesothelioma, endometrial stromal sarcoma, and hepatocellular carcinoma. Metastatic breast carcinoma in the colon can mimic primary signet ring cell carcinoma. When poorly differentiated carcinoma is encountered in the colorectal region, one should always consider metastatic breast carcinoma in women and direct spread from prostate cancer in men.

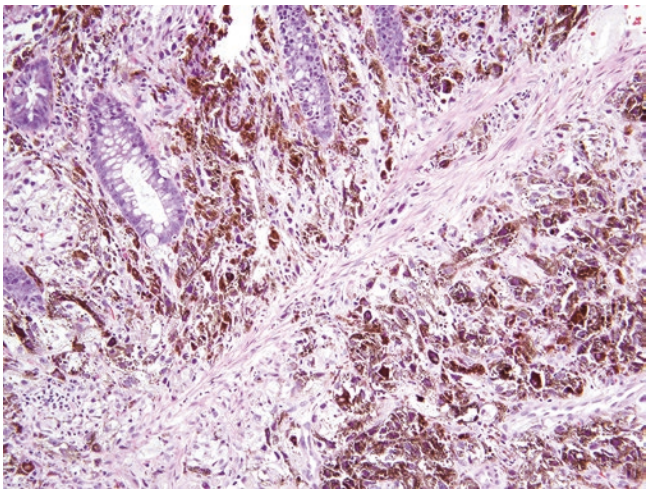

Fig. 3.25 Melanoma. This example shows atypical melanocytes with abundant melanin pigment infiltrating the colonic mucosa and extending into the submucosa

Colorectal Cancer Staging

The purpose of cancer staging is to document the extent of the cancer and is a crucial element to determine the appropriate course of treatment based on the data concerning outcome of patients with similar stage lesions. It also facilitates treatment evaluation, exchange, and comparison of results between different institutions and serves as a basis for cancer research. Several different cancer staging systems are currently used worldwide. However, the tumor node metastasis system (TNM) is the most clinically useful and is discussed here [42]. The American Joint Committee on Cancer (AJCC) and the International Union for Cancer Control (UICC) maintain this system collaboratively. Classification of the tumors by TNM system is based on the size and extent of the primary tumor (T), regional lymph node (N) status, and the presence or absence of the distant metastasis (M) (Table 3.1). Recently, nonanatomic prognostic factors have begun to supplement this cancer staging system.

Most cancers of the colorectal region are staged after surgical resection of the tumor. In this region, the depth of tumor invasion into or beyond the wall of the intestine and invasion or adherence to adjacent organs or structures is also defined by T. The number of lymph nodes involved (N) and presence or absence of distant metastasis (M) are the other features of the TNM staging system. In patients who receive neoadjuvant chemotherapy before the surgical resection a "y" prefix is added to the pathological staging. The TNM staging system for the colorectal region can be used for all the carcinomas arising in this region; however, well-differentiated neuroendocrine tumors of the colon and rectum are staged separately.

The large intestine or colorectum is divided into the cecum, the right or ascending colon, the middle or transverse colon, the left or descending colon, the sigmoid colon and the rectum. The cecum is the blind pouch that connects the terminal ileum to ascending colon and is covered with a visceral peritoneum (serosa). The posterior surface of ascending and descending colon lack the serosa and are in direct contact with the retroperitoneum. The transverse colon is intraperitoneal and is entirely covered by serosa attached to the pancreas by a mesentery. The sigmoid colon is also entirely intraperitoneal and covered by serosa. The rectum is covered by serosa on the anterior side to the middle third and on the lateral walls to upper third. The posterior surface of the rectum lacks serosa. The distal third of the rectum also known as the rectal ampulla has no peritoneal covering. The anal canal extends from the rectum to the anal verge and is 3–5 cm in length.

Lymph nodes are located along the major vessels supplying the colorectal region, adjacent to the colon and also along the arcades of the marginal artery. The number of lymph nodes sampled should be recorded, as it is important prognostically and is associated with increased accuracy in staging the tumor. At least 10 to 14 lymph nodes should be sampled in radical colectomy specimens. However, fewer lymph nodes may be removed or found in patients who have undergone radiation prior to surgical resection. Carcinomas of the colon can metastasize to any organ but the liver and lung are the most commonly affected. Seeding of other segments of the colon, small intestine, and peritoneum can also occur.

Clinical Staging

This staging is based on the medical history, physical examination, and colonoscopy with biopsies. Radiographic evaluations to be done to evaluate extracolonic or extrarectal spread include computed tomography (CT scan of abdomen, chest, and pelvis), magnetic resonance imaging (MRI), and positron emission tomography (PET) or fused PET/CT. Patients with rectal cancer might need a preoperative adjuvant treatment based on the pelvic extent of the disease combined with absence of extra pelvic metastasis. Pelvic MRI alone or with endorectalcoli, pelvic CT, or endoscopic ultrasound can be used to evaluate the pelvic extent of the disease. To evaluate the nodal staging, ultrasound-guided fine needle aspiration (FNA) of the lymph nodes can improve the accuracy. It is important to assign a clinical TNM staging (cTNM) prior to initiating preoperative therapy.

Pathological Staging

Most cancers of the colon and rectum are pathologically staged after surgical resection of the tumor (pTNM). For

patients who were assigned a clinical staging (cTNM) prior to the initiation of the adjuvant therapy, a modified pathological staging is implemented (ypTNM).

Carcinoma in situ (pTis) is defined by cancer cells present within the glandular basement membrane (intraepithelial) or lamina propria (intramucosal) without invasion of the submucosa. Carcinoma in situ of the large intestine has no risk of metastasis. Carcinoma in a polyp is classified with the same principle and was discussed earlier. That is, if the carcinoma cells are within the epithelium or lamina propria, it is considered carcinoma in situ; however, invasion of the submucosa of the polyp head or stalk is considered as pT1. Tumors that invade the muscularis propria are classified as pT2 and when the carcinoma cells invade through the muscularis propria and involve the pericolorectal tissues is assigned to the pT3 category. Tumors that have directly extended and involved the visceral peritoneum, or are histologically adherent to other organs or structures, are classified as pT4. Since tumors in this category have a different prognosis based on the extent of the disease, they are subdivided into pT4a and pT4b. Tumors that directly penetrate the peritoneal surface are classified as pT4a and tumors that are adherent to or directly invade other organs are assigned to the pT4b category.

Lymph node metastasis has been classified as N1 when 1–3 regional lymph nodes are involved by metastatic carcinoma and N2 with 4 or more lymph nodes involved. These two groups have been subdivided into pN1a (1 lymph node involved by metastatic carcinoma) and pN1b (metastasis in 2–3 lymph nodes), pN2a (metastasis in 4–6 lymph nodes), and pN2b (metastasis in 7 or more lymph nodes). These categories have been generated based on the different outcomes within these groups. Tumor deposits or satellite nodules are defined as discrete foci of tumor found in the pericolonic or perirectal fat away from the edge of the tumor with no evidence of residual lymph node tissue. Tumor deposit could be a result of discontinuous spread, venous invasion with extravascular spread, or a totally replaced lymph node.

Metastasis to 1 site, such as only the liver or lung or nonregional lymph nodes, is classified as M1a. Metastases to multiple sites or peritoneal surfaces are recorded as M1b. The absence of metastases or M0 can only be made at autopsy and would be annotated as aM0. If the tumor recurs, the "r" prefix is used for cancer staging (rTNM).

Tumor regression response is the pathological response to perioperative therapy and has a prognostic value. Chemoradiation in rectal cancer leading to complete eradication of tumor determined by pathological evaluation seems to portend a better prognosis than no response or incomplete response. Thus, the specimens from these patients should be thoroughly examined at the primary site, in the regional lymph nodes and for peritumoral satellite nodules or tumor deposits. The degree of response should be recorded and correlated with the prognosis. A 4-point grading system is used to evaluate the tumor regression response. No viable tumor is characterized as complete response (Grade 0), Single cell or small groups of cells is moderate response (Grade 1), residual cancer outgrown by fibrosis is minimal response (Grade 2), and minimal or no tumor kill is poor response (Grade 3).

Circumferential resection margin (CRM) involvement is another prognostic factor that is clinically important. This margin corresponds to any aspect of the colon or rectum that is uncovered by serosal layer and it needs to be dissected from the retroperitoneum or subperitoneum. In the rectum, the peritonalized surface and the nonperitonalized surface can be difficult to identify during the pathological examination of the resected specimen. Thus, surgeons are encouraged to mark the retroperitoneal reflection and the area of the deepest tumor penetration by a suture or a clip. The distance between the closest leading edge of the tumor and the CRM is another prognostic factor. Surgical clearance of 1 mm or less has been associated with local recurrence and should be recorded as a positive margin in rectal samples.

Residual tumor (R) refers to the completeness of the resection and is based on the status of the CRM and also includes any disease observed but not removed during the operation. Complete resection (R0) is designated as a complete resection with all margins uninvolved. Incomplete resection, or R1, refers to the presence of microscopic involvement of the surgical resection margins. Incomplete tumor resection with grossly visible tumor at the resection margin or regional lymph node involvement or incomplete primary tumor resection is characterized as R2.

As medicine advances, it has been feasible to identify isolated tumor cells (ITCs) and molecular node involvement. ITC is defined as a single malignant cell or a few tumor cells in microclusters. These cells usually can be either identified by hematoxylin and eosin (H & E) or the use of immunohistochemistry or molecular testing. Currently, the presence of ITC in regional lymph node is classified as pN0 and its prognostic significance remains unclear.

Some of the other independent prognostic factors include residual disease, histological type, histological grade, serum carcinoembryonic antigen and cytokine level, extramural venous invasion, and vascular invasion by carcinomas. Undifferentiated carcinoma, small cell carcinoma and signet ring cell carcinoma or poorly differentiated carcinoma have less a favorable outcome than other types of carcinoma. However, medullary carcinoma has a more favorable outcome. Submucosal vascular invasion by carcinomas arising in an adenoma is associated with higher risk of lymph node metastasis. Perineural invasion, lymphatic and vascular invasion are also associated with a less favorable outcome.

Another prognostic factor is the presence of a mutation in either codon 12 or 13 of *KRAS* and is associated with lack of response to treatment to anti-EGFR antibodies in patients

with metastatic colorectal carcinoma. Currently, molecular studies are not part of the staging system. However, in the future, evaluation of specific molecular factors might be a component of staging. Moreover, other factors such as age, gender, race, or ethnicity are also important as they may affect the disease outcome and response to therapy.

Colorectal Neuroendocrine Tumor Staging

Well-differentiated neuroendocrine (carcinoid) tumors of the colorectal region are classified according to the neuroendocrine tumors of the GI tract. These tumors in the colorectal region are rare. In the colon, the cecum is the most common location and some might originate from the appendix. Most of the carcinoids arising in the colon are more than 2 cm at the time of the diagnosis and involve the muscularis propria with the overall survival of 33–42%. Rectal carcinoids, on the other hand, have a more favorable outcome with low risk of metastasis. The overall survival for the rectal neuroendocrine tumors is 88.3%. Features predictive of poor outcome are tumor size greater than 2 cm and invasion of the muscularis propria. Neuroendocrine tumor invading lamina propria with the size of 2 cm or less is classified as T1. Tumors less than 1 cm and tumors between 1 cm and 2 cm are further subclassified as T1a and T1b, respectively. Neuroendocrine tumors with invasion of the muscularis propria or size greater than 2 cm with invasion of lamina propria or submucosa are assigned as T2. Stage T3 represents tumors invading through the muscularis propria into the subserosa or into nonperitonealized pericolic or perirectal tissue. Tumors invading peritoneum or other organs are pathological stage T4.

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