Chapter 1
Intraoperative Evaluation of Colorectal Specimens Containing Cancer

Abstract Intraoperative assessment of colon cancer resection specimens may influence immediate surgical management. Indications for evaluation include determining the depth of invasion, presence of serosal penetration, status of margins, and intactness of the mesorectum, when present. Pathologists may also be asked to identify residual carcinoma in neoadjuvantly treated patients, or document the presence of other lesions, such as adenomas, polypectomy sites, or underlying colitis. Estimating the depth of invasion into the colonic wall is best achieved by macroscopic examination in combination with frozen section analysis, whereas detecting serosal involvement may require touch or scrape preparations of the serosal surface. Assessment of margins is most challenging in rectal specimens, particularly when patients have received neoadjuvant therapy.

Keywords Colonic adenocarcinoma · Serosa · Margins · Mesorectum · Neoadjuvant therapy

Introduction

Colorectal carcinoma is the most common carcinoma of the gastrointestinal tract, and more than 150,000 cases are diagnosed in the United States each year [1]. Surgical excision remains
the mainstay of therapy, and pathologic assessment of resection specimens is critically important to the subsequent management of the patient. Pathologic evaluation is necessary to determine the extent of disease, which is the single most powerful predictor of outcome among colorectal cancer patients, and use of the TNM staging system is now standard practice in the United States [2]. Intraoperative assessment of cancer resection specimens may influence immediate management, and indications for evaluation include determining (1) the depth of invasion, (2) presence of serosal penetration, (3) status of margins, and (4) intactness of the mesorectum; identifying residual carcinoma in neoadjuvantly treated patients; and documenting the presence of other lesions, such as polyps, dysplasia in chronic colitis, and tattoos from prior procedures.

Assessing Local Extent of Colorectal Carcinoma

The designations for pathologic tumor stage (pT) describe the deepest point of tumor penetration within the colonic wall [2]. Although final stage classification is deferred to review of permanent sections, pathologists may be asked to provide intraoperative staging information in some cases, such as assessment of apparently superficial lesions, which may be amenable to local excision. Gross assessment of tumor invasion is best achieved by serially sectioning at close intervals, which usually allows one to estimate whether it is limited to the lamina propria, or penetrates the submucosa, muscularis propria, or serosa. Invasive carcinomas appear as tan-white, ill-defined masses that obliterate normal tissue layers of the colonic wall, and the deepest invasion usually occurs in the tumor epicenter [3] (Fig. 1.1).

The term “carcinoma in situ” is generally avoided as a diagnostic category in colorectal neoplasia because it encompasses both intraepithelial carcinoma and tumors that are invasive of the lamina propria but confined to the muscularis mucosae. Most pathologists prefer the term “adenoma with high-grade dysplasia” to describe a neoplastic proliferation confined to the basement membrane
Assessing Local Extent of Colorectal Carcinoma

Fig. 1.1 Invasive adenocarcinoma within a polyp obliterates tissue planes between the mucosa and submucosa and superficially infiltrates the inner layer of the muscularis propria (arrow). The adjacent colonic wall shows a clear demarcation between the superficial mucosa, gelatinous submucosa, and both layers of the muscularis propria.

Cancers that extend into the submucosa (pT1) are generally detected in polypectomy specimens, since these early, often asymptomatic, tumors are identified during screening or surveillance colonoscopy. One potential pitfall to the diagnosis of submucosal invasion is commonly observed in distally located adenomas that are subjected to luminal trauma (Fig. 1.4), or those that have been endoscopically manipulated in a prior procedure (Fig. 1.5). Traumatized adenomas may display epithelial misplacement into the polyp stalk, which has been termed “pseudoinvasion”. The histologic distinction between invasive carcinoma and “pseudoinvasive” epithelium has significant clinical implications, since some patients with malignant polyps require a colectomy, whereas adenomas with epithelial misplacement are treated by polypectomy [5]. A variety of morphologic features help distinguish adenomas with misplaced epithelium from those with invasive adenocarcinoma. Misplaced epithelium resembles that present in the
Intraepithelial adenocarcinoma (high-grade dysplasia) appears as a complex proliferation of fused and cribiform glands (a). The neoplastic cells display loss of polarity, rounded nuclei with heterogeneous chromatin, prominent nucleoli, mitotic figures, and necrotic cellular debris (b).
Fig. 1.3 Intramucosal carcinoma (arrow) typically develops on a background of high-grade dysplasia and consists of irregular clusters on neoplastic glands and single cells limited to the lamina propria (a). Notably, most intramucosal carcinomas do not incite a desmoplastic tissue response (b) and, in fact, the presence of desmoplasia in the lamina propria should alert one to the possibility of submucosal invasion.
Fig. 1.4 Mucosal elements are present in the submucosa of this rectal adenoma. The misplaced crypts are arranged in rounded aggregates associated with inflammation and hemosiderin deposits (a). Although misplaced crypts may rupture and extrude mucin into the submucosa, they are surrounded by a rim of lamina propria (b)
Fig. 1.5  Endoscopic manipulation of colonic adenomas may also induce epithelial misplacement that mimics invasive adenocarcinoma in subsequent excisional specimens. Round aggregates of neoplastic epithelium floating in mucin pools fill the submucosa (a). Unlike invasive carcinomas, however, misplaced epithelium is associated with lamina propria (b).
mucosal component and is accompanied by a distinct rim of lamina propria [6] (Fig. 1.4). The crypts dilate and rupture, inciting an inflammatory response to extruded mucin, and stromal hemorrhage with hemosiderin deposits is common. In contrast, adenocarcinomas grow in an infiltrative pattern, are not accompanied by lamina propria tissue, and incite a desmoplastic stromal response (Fig. 1.6a). Invasive epithelial cells are cytologically atypical with high-grade nuclei, nuclear pleomorphism, and clumped chromatin (Fig. 1.6b).

The distinction between carcinomas that invade, but are limited to, the muscularis propria (pT2) and those that extend into the pericolic or subserosal adipose tissue (pT3) is generally straightforward. The minimal criteria for pT3 classification include an absence of smooth muscle cells of the muscularis propria between the leading edge of the tumor and the pericolic fat [2].

The most problematic aspect of tumor stage assignment is the recognition of pT4 lesions. This stage is subdivided into two categories: pT4a is now defined as serosal penetration, whereas pT4b denotes direct tumor extension into another organ. Serosal penetration produces shaggy, fibrinous serosal adhesions, often with puckering, although some cases may show complete penetration and perforation of the serosal surface (Fig. 1.7a). Serosal involvement may be detected by scraping the serosa and smearing the material on a glass slide or touching a glass slide to the serosal surface and staining the slide with hematoxylin and eosin. Cytology preparations obtained from the serosal surface contain clusters of neoplastic epithelial cells and a background of reactive mesothelial hyperplasia and inflammation (Fig. 1.7b). Serosal penetration is most readily recognized in histologic sections when free tumor cells are present on the serosal surface, or when tumor cells are present at the serosal surface in combination with mesothelial cell hyperplasia or an inflammatory reaction (Fig. 1.8a). The presence of tumor cells within close proximity of a mesothelial inflammatory reaction also predicts decreased survival, despite the apparent lack of tumor cells on the serosa, and most likely represents a tissue response to serosal penetration [7] (Fig. 1.8b). Note that the serosa is not a surgical margin and serosal penetration by tumor does not constitute an incomplete resection.
Fig. 1.6  Invasive adenocarcinomas expand the submucosa. They have an infiltrative appearance, are associated with desmoplasia, and display overtly malignant cytologic features (a). Dissecting mucin pools that contain neoplastic cells may also be present (b).
Fig. 1.7 Colonic adenocarcinomas that penetrate the serosal surface frequently display fibrinous adhesions on the serosa (arrow) directly subjacent to the tumor (a). Serosal penetration is easily documented by creating cytologic preparations from material obtained by touching, or scraping, the serosal surface of the fresh specimen. Neoplastic epithelial cells form cohesive groups on a background of inflammation (b)
Fig. 1.8 Although some pT4a lesions display tumor cells at the serosal surface (a), most show neoplastic epithelium in close proximity to the serosa, in combination with an inflammatory reaction with entrapped mesothelial cells (b)
Assessing Lymph Node Status

Pathologists are rarely asked to assess lymph node status during colon cancer surgery, although one may occasionally receive non-regional lymph nodes for frozen section diagnosis. The regional lymph nodes for colorectal carcinomas are staged as pN0, pN1, or pN2 depending upon the number of lymph nodes involved by carcinoma. Notably, non-regional lymph nodes that contain tumor deposits should be staged as pM1 disease. There is no universal agreement regarding the minimum number of lymph nodes that should be retrieved in order to accurately predict the likelihood of regional node negativity, although removal and examination of at least twelve lymph nodes is generally considered adequate [8]. By convention, rounded tumor nodules within the pericolic fat that are discontinuous with the main tumor mass are considered to represent completely replaced lymph nodes even if no residual nodal tissue is identified.

Evaluation of Resection Margins

A number of problems arise when assessing surgical resection margins on colectomy specimens. Most issues are resolved following close examination of the specimen, review of the radiology report, and direct conversations with the surgeon. Common problems include inadvertent inclusion of the serosa as a resection margin and a failure to recognize the radial resection margin on rectal specimens. Every colonic resection specimen has at least three surgical resection margins: the proximal margin (which should be the ileum on proximal colonic resection specimens), the distal margin, and the radial margin. There is a very high correlation between the impression of margin status on gross examination and histologic findings. Extensive lateral, or submucosal, tumor spread is rare among primary colonic carcinomas, so gross examination of the proximal and distal margins is usually sufficient for margins distant (>3 cm) from the tumor [9]. Tumors present within 1 cm of the margin should be evaluated with perpendicular sections, whereas
parallel (en face) sections may be obtained in cases that display greater tumor clearance. Close margins are commonly encountered among low-lying rectal cancers, since it may be difficult for the surgeon to obtain a wide margin while preserving anal sphincter function [10] (Fig. 1.9). Surgeons may also have a difficult time judging the status of the distal margin in the neoadjuvant setting, since the residual tumor may be small (Fig. 1.10).

The radial margin is a soft tissue resection margin reflecting either transection of the mesentery, or a surgical plane of dissection, and its nature varies depending on the anatomic location of the tumor and the type of surgical specimen obtained. The radial

Fig. 1.9 Invasive adenocarcinomas of the distal rectum may lie in close proximity to the distal resection margin, and thus, surgeons commonly request gross examination of the specimen by pathologists in order to assure adequate tumor clearance. Note the additional presence of a smaller polyp in this resection specimen (arrow)
Fig. 1.10 Bulky, inoperable rectal adenocarcinomas may show a striking response to neoadjuvant therapy, such that the residual lesion appears as an ulcer associated with mural scarring (arrow). Tumors that occur in the most distal rectum may be excised with a narrow margin in order to preserve the anal sphincter. Intraoperative evaluation of close margins should include parallel sections of the tumor in relation to the distal margin.

In contrast, the radial resection margin of the rectum is a circumferential margin because it lies below the peritoneal reflection. The results of several studies have shown that the status of this margin is a powerful predictor of local recurrence and is probably the most important margin on rectal cancer resection specimens [11]. The adipose tissue surrounding the lower two-thirds of the rectum is enveloped by delicate fibroconnective tissue, termed the mesorectum, which may be sharply dissected from adjacent pelvic
structures. The mesorectum begins below the peritoneal reflection in the upper rectum where it is limited to the posterior aspect of the rectum and is continuous with the sigmoid mesocolon [12] (Fig. 1.11). Advances in surgical techniques over the past few decades have led to the widespread practice of total mesorectal excision for rectal carcinoma. In this situation, the surgeon dissects the rectum along the areolar plane outside the mesorectal fascia, such that the rectum, mesorectum, and all regional lymph nodes are removed entirely [13]. This surgical technique decreases the risk of local recurrence and improves overall survival, so pathologists may be called upon to comment on the intactness of the mesorectal envelope in cancer cases [11, 14, 15] (Fig. 1.12). Evaluation

Fig. 1.11 Rectal resection specimens are posteriorly surfaced by the mesorectum, which represents a surgical margin. The mesorectum lies inferior to the peritoneal reflection (arrow) and tapers proximally but is broader in the lower two-thirds of the specimen.
Fig. 1.12 The mesorectum is enveloped within delicate connective tissue that should be smooth when intact (a). Defects in the fibromembranous connective tissue (arrow) should be noted when present (b)
of the mesorectum should always be performed on an unopened specimen in the fresh state. Assessment should include evaluation of smoothness of the mesorectal surface, tissue bulk, and documentation of the presence of defects in the mesorectal fascia [16] (Table 1.1).

Table 1.1  Pathologic classification of mesorectal excision adequacy

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<tr>
<th>Type</th>
<th>Description</th>
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<tbody>
<tr>
<td>Complete</td>
<td>Mesorectum intact and smooth</td>
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<tr>
<td></td>
<td>If present, defects span &lt;5 mm</td>
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<tr>
<td>Nearly complete</td>
<td>Slightly irregular mesorectal fascia</td>
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<tr>
<td></td>
<td>Defects in mesorectal fat do not extend into muscularis propria</td>
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<tr>
<td>Incomplete</td>
<td>Small amount of mesorectal connective tissue</td>
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<tr>
<td></td>
<td>Defects in mesorectal fat extend into muscularis propria</td>
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<tr>
<td></td>
<td>Coning of distal soft tissue</td>
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<td></td>
<td>Irregular radial margin on sectioning</td>
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**Implications of Neoadjuvant Therapy**

Surgeons may request intraoperative consultations on resection specimens from patients who have undergone preoperative radiation and/or chemoradiation, in order to determine the status of the mesorectal and distal margins. Neoadjuvantly treated rectal cancers may appear as a mucosal pucker or shallow ulcer overlying mural fibrosis (Fig. 1.13). In this situation, careful documentation of the status of the radial (Fig. 1.14) and distal margins (Fig. 1.15) with frozen sections is warranted, since residual carcinoma at the margin may be difficult to distinguish from posttreatment scarring by gross inspection alone [10]. Importantly, acellular mucin pools in neoadjuvantly treated patients are considered to represent complete tumor response and are not used to assign pathologic stage (Fig. 1.16).
1.13 A mucosal ulcer near the distal resection margin remains following neoadjuvant therapy. In this situation, perpendicular sections that demonstrate the relationship between the distal margin and residual tumor should be obtained to document adequate resection. The entire ulcer and subjacent colonic wall should be submitted for histologic evaluation, since tumor regression following therapy is an important predictor of outcome.

1.14 Sections obtained from the radial margin of a rectal cancer patient display infiltrative adenocarcinoma associated with mucin pools, enmeshed within fibroinflammatory stroma.
Implications of Neoadjuvant Therapy

Fig. 1.15 Neoadjuvantly treated cancers of the distal rectum are often excised with a narrow margin that may be evaluated with frozen section, when appropriate. In this case, an ulcer was present 1 cm from the distal margin, but frozen section demonstrated microscopic foci of residual cancer at the resection margin.

Fig. 1.16 Tumor regression is often manifest as acellular mucin pools within the rectal wall. Although their presence reflects the pre-treatment extent of the cancer, acellular mucin pools should not be used to assign pathologic stage to neoadjuvantly treated rectal cancers.
References