Pathology of Premalignant and Malignant Disease of the Esophagus

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Introduction

Like most structures of the alimentary canal, the esophagus is a tubular muscular structure that contains a mucosa, submucosa, muscularis propria, and surrounding connective tissue (termed adventitia in the esophagus) (Fig. 3.1). Anatomically, the esophagus extends from the cricopharyngeal muscle, which forms the upper esophageal sphincter, to the lower esophageal junction, where the stomach originates. Histologically, the mucosa consists of a stratified non-keratinizing squamous epithelium, lamina propria and muscularis mucosae. The squamous epithelium sits atop a basement membrane that separates it from the lamina propria. The lamina propria is composed of loose fibroconnective tissue, lymphatic spaces, and capillary vessels. The muscularis mucosa is a thin muscular layer that separates the mucosa from the submucosa. The submucosa is composed of dense irregular fibrovascular connective tissue admixed with scattered mucin-producing glands (esophageal submucosal glands) and ducts, which aid in the passage of food. Deep to the submucosa is the muscularis propria, which is primarily composed of striated muscle in the upper 1/3 of the esophagus, smooth muscle in the lower 1/3 of the esophagus, and a mixture of both in the mid esophagus. Finally, deep to the muscularis propria is the adventitia, a layer of connective tissue and adipose tissue that helps link the esophagus to adjacent structures. The esophagus, unlike most tubular structures of the alimentary canal, lacks a serosa (Fig. 3.1).

Neoplastic transformation can involve any of the cell types found in the esophagus. However, the vast majority of malignant tumors that arise from the esophagus are epithelial in origin. This review will focus on the malignant

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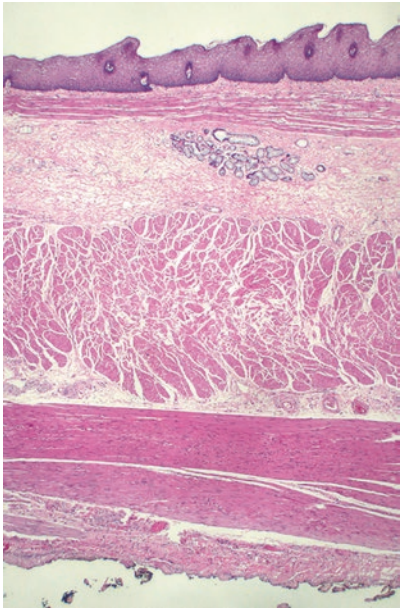

Fig. 3.1 Structural layers of the esophagus (H&E stain). The innermost layer (or tunica) is the mucosa. The mucosa is composed of an epithelial lining (squamous epithelium), the underlying lamina propria (loose connective tissue that supports the epithelium), and the muscularis mucosae (a thin layer of smooth muscle). Deep to the mucosa is the submucosa, which contains more cellular connective tissues, prominent blood vessels (with muscularized arteries), nerve fibers (Meissner plexus), and submucosal mucus (exocrine) glands. A submucosal gland is present in this figure. The thick outer muscular layer is called the muscularis mucosa (or muscularis externa) and in the distal esophagus (as shown in this figure) is composed of an inner circular layer and an outer longitudinal layer of smooth muscle; they layers are separated by the myenteric plexus. Skeletal muscle is admixed with smooth muscle of the muscularis propria in the mid esophagus, and in the proximal esophagus, the outer muscular layers are primarily composed of skeletal muscle. The outermost layer of the esophagus is the adventitia, which is composed of loose connective tissue and helps to anchor the esophagus in place (H&E stain, 20×)

epithelial lesions of the esophagus, namely, adenocarcinoma and squamous cell carcinoma, and their precursor lesions.

Esophageal cancer affects more than 450,000 people worldwide and squamous cell carcinoma is the predominant histologic type [1]. In the United States, Australia, the United Kingdom, and some Western European countries, the incidence of adenocarcinoma is increasing rapidly and now exceeds that of squamous cell carcinoma [1]. The estimated number of new cases of esophageal cancer in the United States in 2019 is 17,650, and the vast majority (13,750) will affect men [2]. Esophageal cancer is estimated to be the seventh leading cause of cancer deaths in men in 2019 in the United States, accounting for 13,020 or 4% of all cancer deaths (following lung, prostate, colorectal, pancreatic, liver cancer and leukemia) [2].

Pathology of Adenocarcinoma and Its Precursor Lesions

Adenocarcinoma typically arises in the distal third of the esophagus and is associated clinically with dysphagia and weight loss. Several risk factors have been described for esophageal adenocarcinoma, including increased age, male gender, white ethnicity, high body mass index, low fruit and vegetable intake, absence of *Helicobacter pylori* infection, the presence of a hiatal hernia, and history of reflux disease [3–10]. Clinical observations and studies in animal models suggest a linear sequence in the development of esophageal adenocarcinoma. Initially, the replacement of the normal squamous epithelium by glandular mucosa (gastric type or intestinal type) occurs secondary to repeated injury from bile and acid reflux. This is followed by dysplastic change within this metaplastic tissue, leading to the development of invasive carcinoma after molecular evolution of the dysplastic epithelium.

Precursor Lesions of Esophageal Adenocarcinoma: Barrett's Esophagus and Barrett's Esophagus-Associated Dysplasia

As recommended by the American College of Gastroenterology in 2016, Barrett's esophagus (BE) "should be diagnosed when there is extension of salmon-colored mucosa into the tubular esophagus extending ≥1 cm proximal to the gastroesophageal junction (GEJ) with biopsy confirmation of intestinal metaplasia (IM)" [11]. Of note, not all professional organizations or countries, including the British Society of Gastroenterology and Japan, require the histologic documentation of intestinal metaplasia [12, 13].

Macroscopically, Barrett's esophagus appears as a well-demarcated area of erythematous or "velvety" mucosa within the squamous-lined tubular esophagus. Histologically, three different types of columnar or glandular metaplasia can be seen on biopsies from patients with "Barrett's" esophagus: (1) gastric cardia-type mucosa (composed of mucin secreting glands); (2) gastric oxyntic (or fundus)-type mucosa composed of parietal cells, chief cells, and mucus secreting cells; and (3) specialized columnar epithelium or intestinal metaplasia containing round, bluish, barrel-shaped cells called goblet cells, often admixed with gastric cardia-type glands (Fig. 3.2). From the pathologist's perspective, the gastric-type mucosa +/− goblet cells seen in the tubular esophagus is indistinguishable from the true gastric mucosa seen in the cardia. Because intestinal metaplasia is commonly seen in the cardia of the stomach in the general population without Barrett's esophagus [14], the American College of Gastroenterology guidelines recommend that biopsies only be taken if there is macroscopic evidence of Barret's esophagus. Biopsies should not be performed in the presence of a normal Z line or a Z line with <1 cm of variability as sampling of gastric cardia with intestinal metaplasia may occur [11]. If the pathologist does not see submucosal glands on a biopsy and does not know what the endoscopist observed at the time of biopsy, a diagnosis of "gastric-type mucosa with intestinal metaplasia" may be rendered instead of a diagnosis of "Barrett's esophagus."

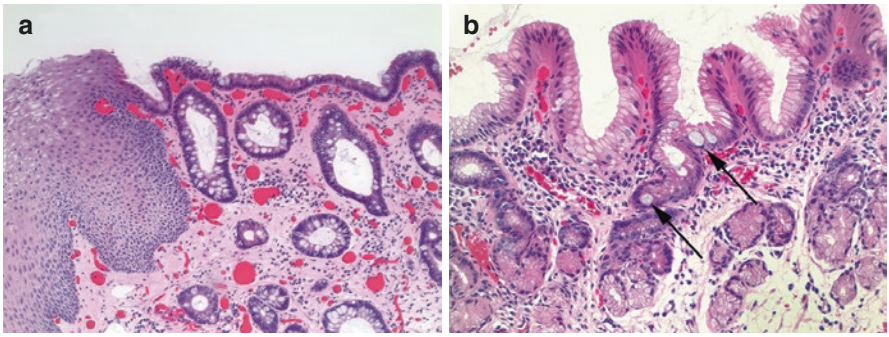

Fig. 3.2 Intestinal metaplasia. (**a**) In this example, the squamous mucosa has been replaced by glandular mucosa of intestinal type. Some intact squamous epithelium is present on the left. All of the glands present on the right contain goblet cells (specialized columnar epithelium or intestinal metaplasia). If this biopsy was obtained ≥1 cm proximal to the GEJ, these findings are consistent with Barrett's esophagus (H&E stain, 100×). (**b**) Gastric-type mucosa with rare goblet cells (focal intestinal metaplasia). Many biopsies from islands of velvety mucosa within the tubular esophagus contain gastric cardiac-type glands. If even one goblet cell is identified, a diagnosis of (focal) intestinal metaplasia can be rendered. As shown in this example, there are about five goblet cells (two are highlighted by arrows) present within gastric-type mucosa. If this biopsy was obtained ≥1 cm proximal to the GEJ, these findings are consistent with Barrett's esophagus (H&E stain, 100×)

It is worth noting that, in addition to designating the presence and/or absence of intestinal metaplasia, pathology reports may detail several other histologic mimics of metaplasia. "Pseudogoblet cells" refer to barrel-shaped gastric foveolar cells that may look like goblet cells on low magnification but have an eosinophilic tinge and do not stain for Alcian blue due to a production of neutral mucins. The term "columnar blues" refers to the identification of mucus cells that contain bluish mucin on the H&E stain and stain positive for Alcian blue but lack goblet cell morphology. The term "multilayered epithelium" may be noted in reports. This finding refers to the identification of an epithelium that contains flattened squamous-appearing cells in the basal layers with an overlying columnar mucus cell layer. Studies have shown that this epithelium can show immunohistochemical features similar to intestinal-type epithelium, indicating that it may represent an early or intermediate phase in the development of intestinal metaplasia and Barrett's esophagus [15]. Finally, pathologists may report the presence of "subsquamous intestinal metaplasia" (also called "buried Barrett's" or "squamous overgrowth"), particularly in endoscopic mucosal resection specimens. Although subsquamous intestinal metaplasia is present in the majority of endoscopic mucosal resection specimens and is no longer felt to be a post-ablative phenomenon, the long-term clinical significance is uncertain and histologic evaluation of squamous-lined mucosa adjacent to areas of BE may be indicated [16, 17].

Once a diagnosis of Barrett's esophagus has been rendered, increased surveillance is indicated at intervals of 3–5 years to monitor for the presence of dysplasia and/or adenocarcinoma. Studies have shown 0.12–0.38% of patients with nondysplastic Barrett's esophagus will develop adenocarcinoma each year [18]; thus

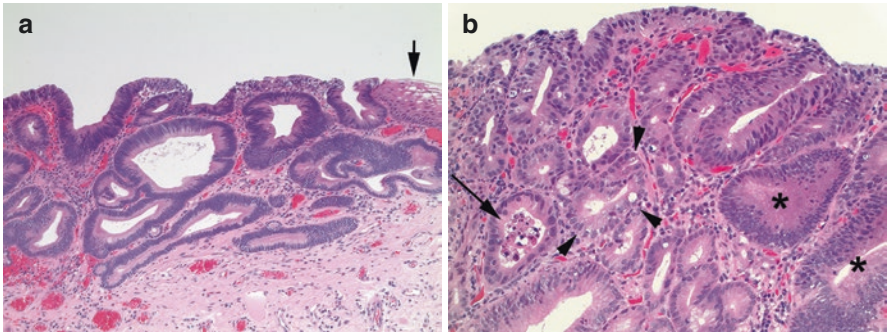

Fig. 3.3 Dysplasia in Barrett's esophagus. (**a**) Low-grade dysplasia. All of the glandular epithelium in this example is involved by low-grade dysplasia; there is a small portion of intact squamous mucosa present (arrow). When dysplasia develops in Barrett's esophagus, the normal goblet cells are often lost because they are replaced by neoplastic epithelial cells that are replicating without normal inhibition and maturation. Characteristic features of low-grade dysplasia include elongation and crowding of the cell nuclei (penicillate nuclei) and increased nuclear-cytoplasmic ratios. These features are typically present from the deep glands all the way to the luminal surface, indicating the lack of maturation. As opposed to high-grade dysplasia, the cell nuclei are still basally oriented (nuclear polarity is maintained), the nuclei are not round, and nucleoli are inconspicuous (H&E stain, 100×). (**b**) High-grade dysplasia. All of the glands in this example show evidence of dysplasia; two glands (∗) show features of low-grade dysplasia, but most of the remaining glands, especially towards the left and towards the luminal surface, show features of high-grade dysplasia. In high-grade dysplasia, there are both cytologic changes and architectural changes. Cytologically, the nuclear-cytoplasmic ratio is increased and the nuclei "round up" often contain prominent nucleoli and lose polarity (several nuclei appear detached from the basal aspect of the cells and are present towards the luminal surface of the glands). Architecturally, the cells proliferate within the lumens of the dysplastic glands, creating cribriform architecture or "gland-within-gland" morphology (arrowheads). Necrotic and apoptotic cells are also often present within glandular lumens (arrow) (H&E stain, 100×)

screening efforts have focused largely on identifying metaplasia in at-risk individuals. Definitive dysplastic change falls into one of two general categories: low-grade or high-grade dysplasia. Features of low-grade dysplasia (Fig. 3.3a) include increased epithelial proliferation characterized by nuclear crowding and minimal distortion of glandular architecture. Mild cytologic atypia and nuclear enlargement, hyperchromasia, stratification, and irregular nuclear contours are also observed. Nuclear-to-cytoplasmic ratios and mitotic counts remain low, basal nuclear polarity is preserved, and in general, the glandular architecture is retained, though there may be increased glandular crowding. Low-grade dysplasia in Barrett's esophagus may resemble the epithelium of a colonic-type adenoma or may be composed of foveolar or gastric-type neoplastic cells. For a diagnosis of low-grade dysplasia, the cytologic atypia should extend to the surface. When surface involvement is not present or cannot be evaluated (for example, due to squamous overgrowth), the term "indefinite for dysplasia" may be used.

In contrast to low-grade dysplasia, high-grade dysplasia (Fig. 3.3b) is characterized by increased cellular atypia and complex architecture. Cells have high

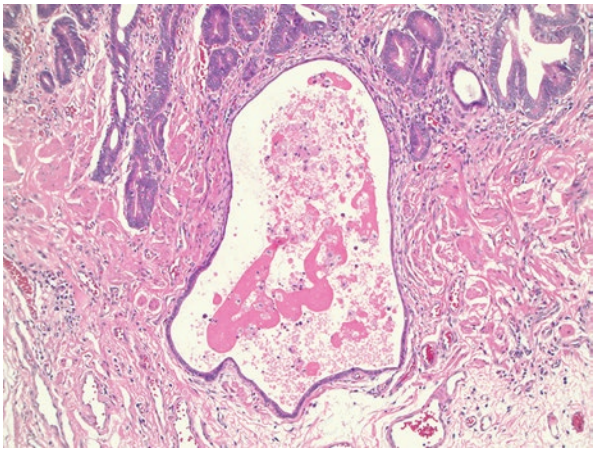

Fig. 3.4 Attenuated gland in the background of high-grade dysplasia. Even though the epithelial lining of the dilated gland is flat and appears bland, the presence of luminal necrotic debris and its presence within muscle bundles make this gland suspicious for attenuated high-grade dysplasia, and it may actually represent early intramucosal adenocarcinoma. If present in the submucosa or within the muscularis propria, attenuated glands with these same histologic features would be diagnostic of invasive adenocarcinoma (H&E stain, 100×)

nuclear-to-cytoplasmic ratios, and there is a loss of basal nuclear polarity with the large often rounded nuclei containing prominent nucleoli. There is also distortion of the normal glandular architecture, often with intraglandular bridging or cribriforming. Cellular or apoptotic debris may be found within glandular lumens. Mitotic activity is evident and often extends beyond the deep proliferative zone and may reach the luminal surface. Deep glands with dilated lumens lined by attenuated epithelium may also indicate the presence of high-grade dysplasia or a lesion of greater clinical significance (Fig. 3.4). In the seventh edition of the AJCC staging manual, the term "Tis" (or "carcinoma in situ") was removed from all epithelial neoplasia of the gastrointestinal tract and was replaced by "high-grade dysplasia" [19]. This distinction is unchanged in the eighth edition of the AJCC staging manual [20]. Since there are some cases of intraepithelial neoplasia that are more atypical than expected for high-grade dysplasia, or cases where definitive invasion cannot be documented, some pathologists may use the term "carcinoma in situ" or "at least high-grade dysplasia." If these terms are noted in the pathology report without an explanatory comment, a discussion with the pathologist may help clarify its meaning in individual cases.

Ancillary studies to aid in the detection of goblet cells and/or dysplasia in Barrett's esophagus are of limited utility at this time. Goblet cells produce acid-rich mucins and thus stain intensely blue with an Alcian blue stain at a pH of 2.5. In addition, the intestinal mucosa stains positive for immunohistochemical stains such as CDX2, villin, and MUC-2 (markers of intestinal differentiation) [21–24]. Although ancillary stains can detect goblet cells and intestinalized epithelium, these stains are not required for a diagnosis of intestinal metaplasia, as the goblet cells can

often be readily identified on the routine hematoxylin and eosin (H&E) stain (Fig. 3.2) [25]. Immunohistochemistry to detect aberrant p53 expression in dysplasia and/or as an indicator of malignancy risk may be performed by pathologists; however, its utility in complementing routine histology remains uncertain. Some studies indicate that p53 may be useful in the identification of dysplasia and malignant progression [26, 27]. Immunohistochemistry for p53 is used occasionally by pathologists as an adjunct to diagnosis in select cases. However, based on the review by the Rodger C. Haggitt Gastrointestinal Pathology Society, routine use of ancillary studies is not recommended for the diagnosis of Barrett's esophagus, dysplasia in Barrett's esophagus, or for the final determination of high risk of malignant progression [25]. Further larger prospective studies are needed before p53 or other biomarkers are recommended for this purpose [25].

As recommended by the American College of Gastroenterology, a diagnosis of dysplasia of any grade within Barrett's esophagus should be rendered after review by two separate pathologists, and ideally, this review would include at least one pathologist with expertise in gastrointestinal pathology [11]. This is due to the high interobserver variability seen in the diagnosis of dysplasia in Barrett's epithelium. Such variability is seen most often in the categories of low-grade dysplasia and indeterminate for dysplasia [28]. Variability is also seen between academic and community-centered practices. Recent studies have demonstrated a large percentage of diagnoses of low-grade and indeterminate for dysplasia rendered at community-based practices are downgraded when reviewed by a pathologist with gastrointestinal pathology training [29–31]. However, a pathologist with this specialized gastrointestinal training may not always be available in all settings, and in some settings a second pathologist may not be available for additional review. Therefore, it is important to communicate with the pathologist to determine how to approach these instances in which a new diagnosis of dysplasia in Barrett's esophagus is made.

Esophageal Adenocarcinoma

Once adenocarcinoma develops, it can be managed by different modalities. Small early lesions may be amenable to endoscopic mucosal resection. Surgery +/− neoadjuvant therapy is the treatment of choice for more deeply invasive or more advanced lesions. Grossly, esophageal adenocarcinoma often appears as an infiltrative mass in the distal third of the esophagus, although fungating, polypoid, and flat growths can be seen (Fig. 3.5a). Histologically, adenocarcinoma is characterized by invasion beyond the basement membrane. Invasion beyond the basement membrane into the lamina propria of the esophagus is a significant development, as it allows access to lymphatic channels that are not found in other areas of the alimentary track, such as the colon. Hence, as per the AJCC staging recommendations, early esophageal adenocarcinoma is staged as either T1a (intramucosal invasion) or T1b (submucosal invasion) [20].

In pathology, most early adenocarcinomas are evaluated on either small biopsies or endoscopic mucosal resection (EMR) specimens. Determining the depth of

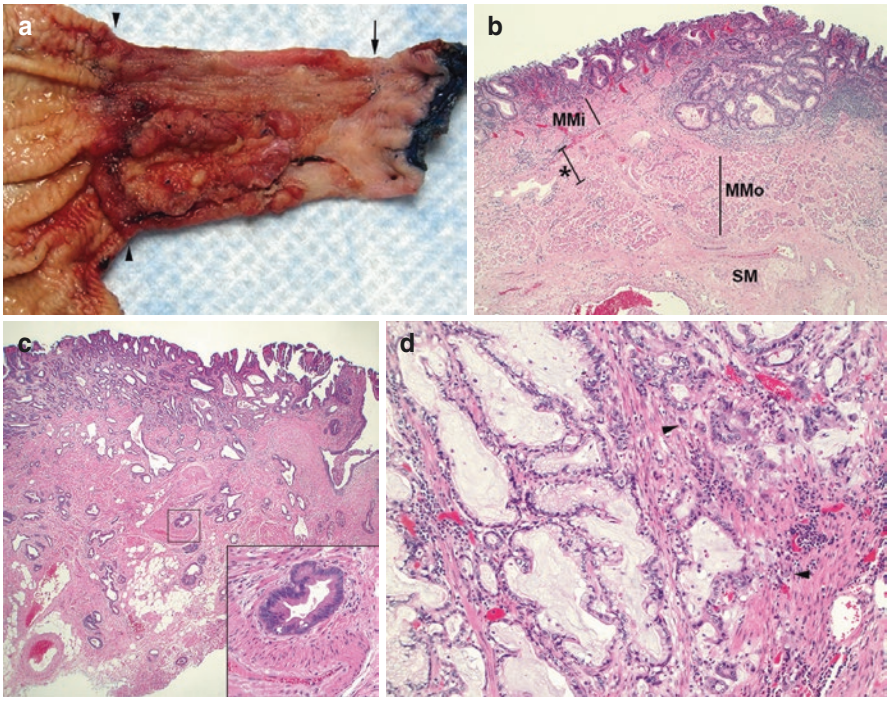

Fig. 3.5 Adenocarcinoma. (**a**) Esophagogastrectomy specimen with adenocarcinoma. The stomach, with normal rugal folds, is towards the left, while the inked squamous-lined esophageal margin is towards the right. The arrowheads highlight the esophagogastric junction. The majority of the esophagus is lined by velvety-appearing mucosa, which represents Barrett's esophagus. The arrow indicates the squamocolumnar junction, which is several centimeters from the esophagogastric junction. Within the distal esophagus and within Barrett's segment, there is an exophytic mass that is an adenocarcinoma. This patient did not receive neoadjuvant therapy prior to the resection. (**b**) Intramucosal adenocarcinoma. A small focus of adenocarcinoma is invading with a pushing border into the space (∗) between the two layers of the duplicated muscularis mucosae, making this a pT1a adenocarcinoma. Even though this tumor has invaded through the internal layer of the muscularis mucosae (MMi), it has not invaded through the outer (duplicated) layer (MMo), so this is not invasive into the submucosa (SM) (H&E stain, 40×). (**c**) Well-differentiated adenocarcinoma. This endoscopic mucosal resection specimen shows dysplasia towards the luminal surface and glandular structures "dripping" through the muscularis mucosae into the submucosa. On low magnification (H&E stain, 20×), there is no obvious desmoplastic response. On high magnification (inset, H&E stain, 200×), if taken out of context, the malignant gland could represent a dysplastic gland, but its presence in the submucosa (as noted by the large muscular artery adjacent to the gland) indicates that this is invasive adenocarcinoma present in the submucosa. (**d**) Invasive adenocarcinoma. This example is primarily well differentiated as most of the malignant cells are forming glands. There is not much desmoplasia in this example, but the malignant glands are present within the muscularis propria and the smooth muscle fibers can be seen in the background stroma. Compared to high-grade dysplasia, there is a bit more cytologic and nuclear atypia in this example of adenocarcinoma, including more nuclear pleomorphism, as well as irregularly shaped glands (upper right corner) and some small nests and single cell infiltration (arrowheads) (H&E stain, 100×)

invasion can be challenging on such specimens because of a phenomenon known as duplication of the muscularis mucosae. For unknown mechanistic reasons, in areas of intestinal metaplasia (Barrett's esophagus), a new internal layer of the muscularis mucosa is created (Fig. 3.5b) [32, 33]. In order to become a T1b lesion, an adenocarcinoma arising in these areas needs to invade through three layers: the "new" superficial muscularis mucosae, the loose connective tissue between the two layers, and then the deep or true muscularis mucosae. Hence, true submucosal invasion is difficult to determine on small biopsies or superficial EMR specimens.

Since EMR specimens are small but contain pertinent information, recommendations for the handling of such specimens have been reported [34]. Ideally, fresh specimens should be pinned to cork or foam prior to formalin fixation. Photographic documentation is recommended. Then the entire specimen should be submitted for histologic evaluation. In addition to reporting the main pathologic findings (dysplasia or intramucosal carcinoma), pathologists must comment on the status of the lateral (mucosal) and deep margins. If an adenocarcinoma is present, the depth of invasion must be assessed. As noted above, the pitfall of overcalling a T1a lesion as a T1b tumor due to a duplicated muscularis mucosae must be avoided. The AJCC further subdivides both intramucosal (m1, m2, and m3) and submucosal (sm1, sm2, sm3) invasion [20]. Although the AJCC does not take into account the different layers of the duplicated MM, other studies have [35]. Hence, pathologists should attempt to describe the specific depth of invasion, for example, "intramucosal adenocarcinoma, invasive into the superficial muscularis mucosal layer." However, only the depth of mucosal invasion may be discernable on biopsies or small EMR specimens. Determining the different levels of submucosal invasion (i.e., sm1, sm2, sm3) is not practical in biopsies or small EMR specimens where the outer limit of the measurement (border of the muscularis propria) is absent. However, given the high risk of nodal involvement with submucosal invasion, the precise depth of invasion on these types of specimens should not alter patient management.

Grading of adenocarcinoma has prognostic significance and falls into one of three categories: well (G1), moderately (G2), or poorly (G3) differentiated; undifferentiated tumors are uncommon, often cannot be subtyped as squamous or glandular, and are considered to be grade 4 (G4) tumors. The importance of accurate tumor grading is critical for clinical management, as AJCC guidelines for clinical staging of adenocarcinoma integrate tumor grade into the algorithm for determining clinical stage (Table 3.1) [20]. For example, a well or moderately differentiated T2 N0 M0 tumor is stage IC, while a poorly differentiated T2 N0 M0 tumor is stage IIA. For adenocarcinoma, grading involves determining the percent of tumor that is composed of glands: well-differentiated tumors contain >95% glands, moderately differentiated tumors contain 50–95% glands, and poorly differentiated tumors display <50% glandular architecture [36]. When tumors contain areas of multiple grades, the highest grade is documented. Histologically, well-differentiated tumors are composed of glands with irregular shapes or profiles (often with focal cribriform formation) lined by cuboidal to columnar cells with mild to moderate atypia. Not all adenocarcinomas illicit a desmoplastic response. In the absence of desmoplasia, a well-differentiated

Table 3.1 Influence of grade on clinical stage for esophageal adenocarcinoma and squamous cell carcinoma

T	Grade	Clinical stage
Adenocarcinoma		
T1	1 or X	IA
T1a	2	IB
T1b	1, 2 or X	IB
T1	3	IC
T2	1 or 2	IC
T2	3 or X	IIA
Squamous cell carcinoma		
T1a	1 or X	IA
	2 or 3	IB
T2	1	IB
	2, 3 or X	IIA
T3	1	IIA
	2, 3 or X	IIB

Note: For all entries above, the N stage is N0 and the M stage is M0. Grade 1 is well differentiated, grade 2 is moderately differentiated, and grade 3 is poorly differentiated

adenocarcinoma could be easily misdiagnosed as dysplasia if assessed out of context. Hence, even the most bland-appearing glands are adenocarcinoma if they are present in the submucosa or muscularis propria (Fig. 3.5c). Esophageal adenocarcinomas are often well-to-moderately differentiated (Fig. 3.5d). As tumors become more poorly differentiated, a more sheetlike appearance is identified. Signet-ring cells, single infiltrating cells, and/or wildly atypical tumor cells may be present. Often, poorly differentiated tumors elicit a strong desmoplastic response from neighboring stromal cells. When dealing with a poorly differentiated adenocarcinoma on biopsy specimens, metastatic disease should be considered and excluded, either clinically or immunohistochemically (for example, immunostains for breast markers could help diagnose metastatic breast carcinoma). Unfortunately, there are no immunomarkers that are diagnostic of esophageal adenocarcinoma, although most tumors express CK7 and may express CDX2 and CK20.

Pathology of Squamous Cell Carcinoma and Its Precursor Lesions

The development of invasive squamous cell carcinoma in the esophagus, like squamous cell carcinoma at other sites, is thought to arise from progression of a dysplastic epithelium [37, 38]. The sequence of events leading to squamous dysplasia and squamous cell carcinoma is ill defined. However, it is clear from association studies that chronic irritation, inflammation, and/or genetic factors are contributory. In the United States, several risk factors for squamous cell carcinoma have been documented including alcohol consumption, smoking, lye exposure, hot beverage consumption, exposure to nitrates/nitrosamines, male gender, increased age (with peak incidence in seventh decade), as well as African-American race [39, 40].

A synergistic relationship between alcohol consumption and smoking is well documented, though the precise molecular basis for this association remains uncertain [41]. Medical conditions that predispose the esophagus to chronic irritation, including achalasia and diverticula, are associated with increased risk [42]. Non-epidermolytic palmoplantar keratoderma, a disease associated with hyperkeratosis as a result of keratin gene mutations, is also associated with increased risk and patients are often counseled about increased screening [43]. Finally, with the rather recent acknowledgment that human papillomavirus (HPV) infection is a risk factor for oropharyngeal squamous cell carcinoma, the association between HPV infection and esophageal squamous cell carcinoma has been studied. However, while HPV has been shown to be causative in some cases of esophageal disease in high prevalence areas, there are cases that do not show any association with HPV infection [44]. Hence the development of esophageal squamous cell carcinoma appears to be multifactorial.

Precursor Lesions of Esophageal Squamous Cell Carcinoma

Squamous dysplasia is uncommonly detected when there is not a concomitant carcinoma. Endoscopically, dysplastic epithelium may appear friable and erythematous. Because squamous dysplasia is difficult to observe grossly, special stains may be used to highlight areas concerning for dysplasia, including toluidine blue (a basic dye which binds to nucleic acids and highlights areas with increased nuclear content common in dysplasia) and Lugol's iodine stain (which highlights areas with decreased glycogen content seen in dysplasia). Cytologically, dysplastic cells are indistinguishable from those observed in invasive disease, but they are confined to the epithelium by an intact basement membrane. Histologic features of squamous dysplasia include increased nuclear-to-cytoplasmic ratios, nuclear hyperchromasia, and pleomorphism. Premature keratinization (e.g., dyskeratosis) may be observed in the cytoplasm and an increased mitotic activity is typically present. Cells lose their polarity and may display increased crowding. Squamous dysplasia is separated into two categories: low-grade intraepithelial neoplasia (LG-IEN), which includes mild and moderate dysplasia, and high-grade intraepithelial neoplasia (HG-IEN), which includes severe dysplasia and squamous cell carcinoma in situ (WHO 5th ed.) [45]. With LG-IEN, the above neoplastic changes are generally confined to the lower third of the epithelium, whereas with HG-IEN, these features extend to the surface. As with adenocarcinoma, high-grade lesions portend a higher risk towards developing invasive disease.

Esophageal Squamous Cell Carcinoma

Squamous cell carcinoma typically arises as a mass in the middle third of the esophagus, although an estimated 30% of cases can arise in the distal third of the esophagus [46]. Typically, squamous cell carcinoma appears as a firm, white flat mucosal

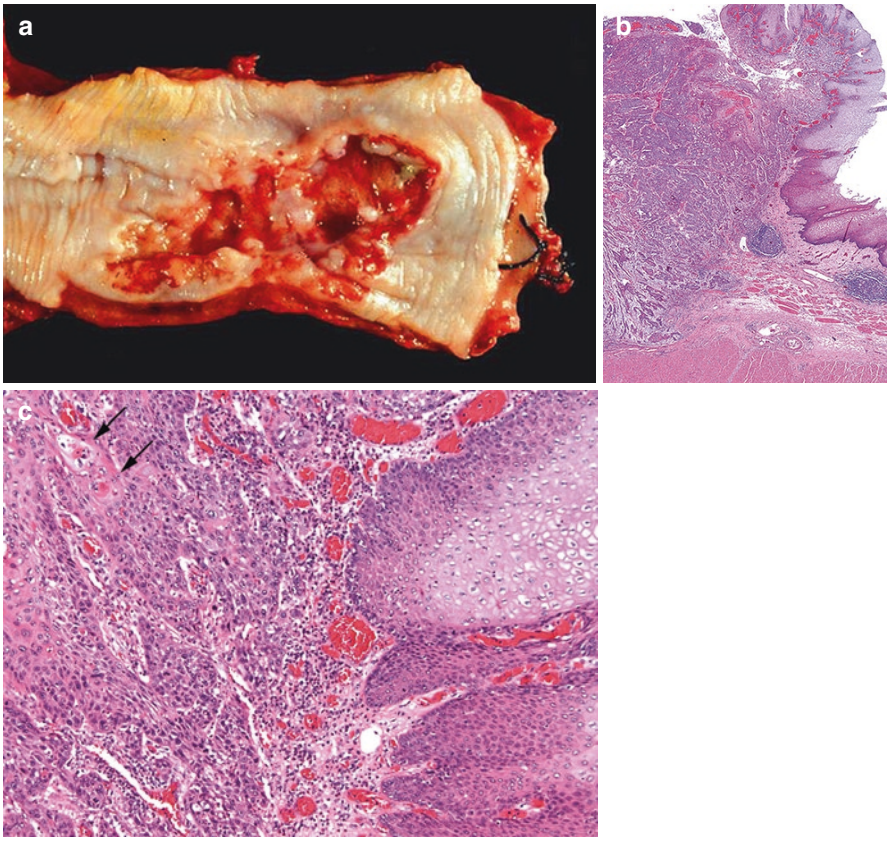

Fig. 3.6 Squamous cell carcinoma. (**a**) Esophagogastrectomy specimen (only the esophagus is shown with the esophageal margin towards the right). A large ulcerating tumor with heaped-up edges is present within the squamous-lined esophagus. (**b**) Histologically, this low-magnification image shows the invasive squamous cell carcinoma (towards the left) undermining the squamous mucosa (to the right) and invading down into the muscularis propria (H&E stain, 20×). (**c**) Histologically, at higher magnification, the invasive squamous cell carcinoma on the left is attempting to recapitulate normal squamous epithelium (present on the right). Although there is a suggestion of keratin formation within the tumor (arrows), overall this tumor is moderately differentiated (H&E stain, 100×)

lesion, as an exophytic ulcerated mass or as polypoid projections, that latter presentation being associated with a spindled morphology (Fig. 3.6a). Surface ulceration is often present.

Squamous cell carcinomas are graded as either well (G1), moderate (G2), or poorly (G3) differentiated based on their ability to recapitulate squamous epithelial cells (Fig. 3.6b,c); undifferentiated tumors are uncommon, often cannot be subtyped as squamous or glandular, and are considered to be grade 4 (G4) tumors. As with adenocarcinoma, accurate grading of specimens is critical as AJCC guidelines for clinical staging likewise integrate tumor grade into the algorithm for tumor staging

(Table 3.1); of note, the change in clinical stage occurs between well and moderately differentiated, which is different than for adenocarcinomas, where the cutoff is between moderately and poorly differentiated [20, 36]. Well-differentiated lesions will display the nuclear features of dysplastic change and maintain an increased nuclear-to-cytoplasmic ratio, but will have intracellular bridges and/or bright eosinophilic swirls of keratin (e.g., keratin pearls). Often, an intense desmoplastic reaction is observed in the surrounding stroma. As lesions become less differentiated, more varied histologic features will be observed, often with sheets or nests of basophilic cells. Frequently, single cell invasion can be identified. Rare intercellular bridge and keratin pearl formation may be identified, but are not abundant. In poorly differentiated tumors, immunohistochemical stains for CK5/6, p63, and p40 may prove useful. In the case of undifferentiated squamous cell carcinoma, they prove diagnostic.

Assessment of Specimens

Beyond indicating histologic subtype and tumor grade, special care must be taken to detail additional factors that will impact patient prognosis. These include margin status; tumor size and location; depth of invasion (e.g., pathologic T stage); presence of lymphatic, vascular, or perineural invasion; and lymph node status (if lymph nodes are present in the specimen). Additionally, if neoadjuvant therapy is administered, assessment of therapy effect is warranted.

Tumors that are not amenable to endoluminal therapy will likely require partial or complete esophagectomy, which should include a portion of the proximal stomach and the adjacent soft tissue and/or lymph nodes. When received in the pathology laboratory, the entire radial/adventitial margin is inked allowing for assessment of margin status. The serosal surface of the stomach should also be inked (typically, with distinct colors) to allow for orientation and to assess for serosal involvement. Once inked, the specimen is opened longitudinally. Appropriate sections should include the esophageal and gastric margins, full thickness sections of the lesion at the point of greatest depth of invasion (with inked adventitial margin), and representative sections of tumor in relation to the proximal (esophageal) and distal (e.g., gastric) mucosa. For cases with preoperative chemotherapy and/or radiation therapy, the lesion typically appears as an excavated scar and should be entirely submitted to allow for assessment of treatment effect. All lymph nodes identified by either palpation of direct visualization should be submitted and evaluated for metastatic disease. The National Comprehensive Cancer Network currently specifies that at least 15 lymph nodes should be examined after esophagectomy [47]. Regional lymph nodes extend from periesophageal cervical nodes for the cervical esophagus to celiac lymph nodes for the distal esophagus. Anatomic dissection should include upper mediastinal and perigastric lymph nodes if possible (in addition to periesophageal lymph nodes) as recent anatomic and clinical studies suggest that submucosal lymphatic vessels connect longitudinally to the superior mediastinal and the paracardial lymphatics, while lymphatic routes to periesophageal nodes originate from the muscle layer [47].

Current staging guidelines for esophageal carcinoma follow the TNM staging system detailed in the eighth edition of AJCC guidelines published in 2017 [20]. This system applies to those lesions which arise primarily in the esophagus, including those involving the esophagogastric junction with or without proximal stomach involvement. As mentioned before, unlike most clinical staging systems, the clinical staging of esophageal carcinomas integrates tumor grade for both squamous and adenocarcinoma (Table 3.1). Adenosquamous carcinomas (e.g., those lesions with both glandular and squamous differentiation) are staged according to squamous protocols. Primary pathologic tumor staging (e.g., pT staging) involves assessing the depth of invasion and is the same for both squamous and adenocarcinoma. High-grade lesions confined to the epithelial basement membrane are classified as pTis (this includes high-grade dysplasia in Barrett's esophagus and HG-IEN squamous dysplasia). pT1 lesions include those lesions in which lamina propria, muscularis mucosae, or submucosal invasion can be demonstrated, with invasion into the lamina propria or muscularis mucosae being classified as pT1a and invasion into the submucosa classified as pT1b. Adventitial involvement is classified as pT3 lesions, while involvement of adjacent structures (e.g., aorta, pleura, pericardium, diaphragm) is classified as pT4. The eighth edition of the AJCC guidelines split pT4 lesions into two stages: those that are resectable (pT4a) and those that are deemed unresectable (pT4b). Finally, lymph node involvement is divided into 5 categories: pNX, pN0, pN1, pN2, or pN3. pNX is used for specimens in which lymph nodes cannot be assessed or were not removed (e.g., EMR specimens, by default, will not have lymph nodes). pN1 designates involvement in 1–2 lymph nodes, while pN2 represents involvement in 3–6 lymph nodes. Involvement of 7 or more lymph nodes is classified as pN3. Extranodal extension, in which metastatic deposits erode the lymph node capsule and extend into the perinodal space, is associated with poor prognosis and may be indicated in reports when present.

Finally, since esophageal cancer has a poor 5-year survival rate of only 17% for all stages [48], some patients are offered neoadjuvant chemotherapy and/or radiation therapy prior to surgery. Similar to other tumor sites, such as rectum and pancreas, this approach has several theoretical benefits. Neoadjuvant therapy may (1) improve symptoms, such as dysphagia, (2) downstage the tumor with the hope of increasing resection rates, (3) treat micrometastatic disease that is not detected on imaging studies, and (4) indicate the biologic behavior of the tumor by its response to treatment that may help guide further therapy [49].

In the TNM classification, specimens that have received neoadjuvant chemotherapy and/or radiation therapy should be designated with a "y" prefix (e.g., ypT3, ypN2). Studies have shown that the pathologic responses in the tumor to primary therapy are important predictors of local recurrence and long-term survival [50–52]. The College of American Pathologists' Protocol for the Examination of Specimens from Patients with Carcinoma of the Esophagus recommends the reporting of response to prior chemotherapy or radiation therapy (www.CAP.org). Although other grading systems exist [50, 52, 53], the CAP assigns response to one of four tumor regression grades. According to this system, those specimens in which no

viable cancer cells can be found and are suggestive of complete response are classified as grade 0. When single cells or small groups of cancer cells are identified, response is deemed "moderate" and is given a tumor regression grade of 1. Minimal response (e.g., grade 2 response) represents those specimens in which residual cancer shows extensive fibrosis, while grade 3 lesions (e.g., poor response) represent those lesions in which minimal tumor lysis is observed and extensive residual cancer remains. Sometimes sizable pools of acellular mucin are observed after treatment; importantly, these acellular pools of mucin should not be interpreted as residual disease.

HER2-Neu Testing

In October 2010, the FDA granted approval for trastuzumab for the first-line treatment of HER2+ metastatic esophagogastric adenocarcinoma in combination with cisplatin and capecitabine or 5-fluorouracil. This approval followed the publication of the results of the ToGA trial that showed a 2.7-month prolongation of medial overall survival in patients with advanced gastric, esophageal, or esophagogastric adenocarcinomas that overexpressed HER2 [54]. In 2016, the College of American Pathologists, the American Society for Clinical Pathology, and the American Society of Clinical Oncology issued guidelines for HER2 testing and clinical decision making for patients with esophageal or gastroesophageal junction adenocarcinoma [55]. These guidelines recommend assessment of HER2 overexpression in patients with locally advanced, recurrent, or metastatic adenocarcinoma of the esophagus/gastroesophageal junction. Per these recommendations, assessment of HER2 overexpression can be performed on biopsy or resection specimens prior to initiation of treatment with trastuzumab. The use of cell blocks prepared from cytologic preparations to assess for HER2 overexpression is also deemed acceptable, though not ideal. HER2 assessment can also be performed in metastatic lesions if needed. It is suggested that the tissue block containing the lowest grade of tumor should be used for HER2 assessment. The recommendations further state that appropriately validated HER2 immunohistochemistry (IHC) be performed initially. Of note, the scoring system for HER2 positivity in gastric or gastroesophageal junction cancer is different from the scoring used in breast cancer [54, 55]. Cases showing 3+ IHC expression are interpreted as positive, 2+ expression as equivocal, and both 1+ and 0 expression as negative. According to the 2016 recommendations and NCCN guidelines, samples with equivocal (2+) IHC expression must then be examined by HER2 in situ hybridization [55, 56]. Cases with 3+ overexpression by IHC (Fig. 3.7a) or cases showing ISH positivity (a HER2:CEP17 ratio of ≥ 2) are considered positive. These patients are then eligible for combination chemotherapy and trastuzumab. It is important to realize, though, that only a relatively small number of esophageal adenocarcinomas overexpress the HER2 protein on the surface of their cells; the positivity rate ranges from 17% to 22% [57].

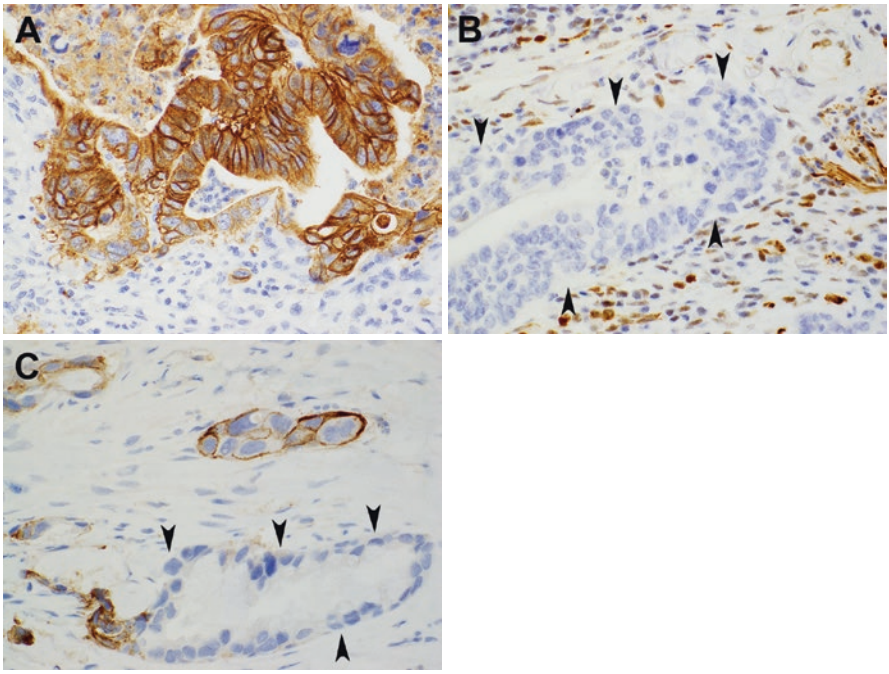

Fig. 3.7 Her2, MLH1 and PD-L1 testing by immunohistochemistry. (**a**) This is an example of Her2 overexpression in esophageal adenocarcinoma. There is strong, complete basolateral membranous (brown) staining in ≥10% of the tumor cells (HER2 immunohistochemical stain, 400×). (**b**) This is an example of loss of MLH-1 in adenocarcinoma. The background inflammatory and stromal cells show scattered nuclear staining. However, the cells within the malignant gland (arrowhead) show complete loss of nuclear staining for the mismatch repair protein. PMS2 would also be lost in this case (MLH-1 immunohistochemical stain, 400×). (**c**) This is an example of PD-L1 expression in adenocarcinoma of the gastroesophageal junction. There is strong membranous (dark brown) staining of some tumor cells, as well as negative staining in tumor cells (arrowheads), with a resultant combined positive score (CPS) of ≥1 (PD-L1 immunohistochemical stain, 400×)

Microsatellite Instability Testing

In May 2017, the FDA granted accelerated approval for pembrolizumab, a PD-1 inhibitor, for the treatment of unresectable or metastatic solid tumors that are microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR). This includes solid tumors that have progressed despite previous chemotherapeutic treatment and for which there is determined to be no other adequate therapeutic treatment option. Microsatellite instability and/or mismatch repair deficiency can occur at relatively high frequencies in colorectal, gastric, esophageal, and pancreatic adenocarcinomas and at lower frequencies in endometrial, bladder, ovarian, and other carcinomas [58, 59]. This approval by the FDA is unprecedented and unique because treatment parameters are not defined as site or tumor specific, but are rather based

on the presence of a molecular abnormality in potentially any tumor type in any location. Based on the results of five single-arm multicohort multicenter trials (KEYNOTE-016, KEYNOTE-164, KEYNOTE-012, KEYNOTE-028, and KEYNOTE-158), the NCCN guidelines recommend pembrolizumab for second-line or subsequent therapy for MSI-H or dMMR for esophageal and esophagogastric junction adenocarcinomas [56, 58, 60].

Microsatellite instability testing can be performed either molecularly, to detect patterns of microsatellites in key genes, or immunohistochemically, to detect loss of protein expression as a surrogate marker of an abnormally functioning gene. Microsatellites are simple (1 or more base pair) units that may be repeated up to 100 times and are scattered throughout the genome. Due to their redundancy, errors, such as DNA slippage, can occur during DNA replication. Mismatch repair genes play a critical role in the identification and correction of these errors. Failure of the mismatch repair apparatus leads to persistence of errors and an alteration in the length of a microsatellite sequence. Persistence of such errors leads to frameshift mutations with loss of the normal function of the involved genes, which can lead to tumorigenesis. MSI is defined as a change of any length due to either insertion or deletion of repeating units in a microsatellite within a tumor when compared to normal tissue. MSI can be detect by PCR using a validated panel of microsatellites or as part of a validated next-generation sequencing panel.

Currently, many pathology laboratories routinely use immunohistochemistry as the test of choice to determine the microsatellite status of a tumor. The DNA mismatch repair system requires the cooperation of many genes, including *MLH1*, *PMS2*, *MSH2*, and *MSH6*. Biochemically, the MSH2 protein recognizes and binds directly to the mismatched DNA sequence and then forms a heterodimer with MSH6. Binding of a second heterodimer, MLH1 and PMS2, is needed for proper function of the MMR complex to adequately excise and repair the mismatched nucleotides. Intact staining of all four proteins indicates that the tumor is mismatch repair protein proficient and therefore microsatellite stable. Loss of staining for one or two paired proteins indicates that the tumor is dMMR and therefore MSI (Fig. 3.7b).

PD-L1 Testing

In September of 2017, the FDA granted accelerated approval of pembrolizumab for the treatment of recurrent locally advanced or metastatic, gastric, or gastroesophageal junction adenocarcinoma that expresses PD-L1. Patients are eligible for treatment as a third-line or subsequent therapy if disease progression occurs after two attempts with fluoropyrimidine- and platinum-containing chemotherapy and/or HER2/neu-targeted therapy [56, 61]. The FDA-approved immunohistochemical stain for the determination of PD-L1 status is the 22C3 pharmDx antibody kit by Dako. Expression of PD-L1 within adenocarcinoma is determined by calculation of a combined positive score (CPS), which is assessed by positive membranous staining of PD-L1 within tumor cells and tumoral or peri-tumoral lymphocytes and

macrophages; a total number of 100 tumor cells must be present. This total is divided by the total number of cells examined, then divided by 100. A CPS of ≥1 is considered positive (Fig. 3.7c) and the tumor eligible for third-line treatment with pembrolizumab [56]. Initial diagnostic tissue prior to the initial attempts of treatment can be used to evaluate for PD-L1 expression. However, additional tissue can be obtained if indicated.

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