Chapter 2
Intraoperative Evaluation of Hepatic Biliary Lesions

INTRODUCTION
Most hepatic biliary lesions are benign and incidentally discovered during procedures unrelated to the liver. Benign bile duct proliferations are gray–white subcapsular nodules that are often multiple, so it is not surprising that their detection results in more liver frozen sections than any other finding. In contrast, a diagnosis of intrahepatic cholangiocarcinoma is almost always known prior to surgery. Thus, diagnostic frozen sections are rarely performed. Cholangiocarcinomas that involve the liver occur either in the proximal extrahepatic bile ducts and directly extend into the liver (hilar cholangiocarcinoma or Klatskin tumor) or they arise from the intrahepatic biliary tree. Intrahepatic cholangiocarcinomas are subclassified in two broad groups based on their anatomic location in the porta hepatis and/or large ramifying ducts (central cholangiocarcinoma) or smaller ducts (peripheral cholangiocarcinoma). Intraoperative consultations for cholangiocarcinoma are usually limited to bile duct margin evaluation on tumors that involve the perihilar region, although surgeons may request evaluation of parenchymal margins for peripheral cholangiocarcinomas. In the former situation, frozen sections are obtained from the main hepatic resection specimen or separately submitted segments of extrahepatic bile duct. The purpose of this chapter is to discuss the following: (1) the histologic features of benign bile duct lesions, (2) criteria that aid their distinction from carcinoma, and (3) issues related to margins of resection for malignant biliary-type tumors that involve the liver. Extrahepatic biliary neoplasia is discussed in further detail in Chapter 6.
BENIGN BILE DUCT LESIONS OF LIVER

Bile duct hamartomas (von Meyenberg complexes) and bile duct adenomas (biliary adenomas) are common, incidentally discovered lesions that generally escape preoperative detection because they are too small to be seen radiographically. Both types of lesion occur as solitary, or multiple, white nodules that span only a few millimeters and are more numerous under the capsule (Fig. 2.1). Their multifocal nature raises concern for possible metastatic disease and they are frequently submitted for intraoperative evaluation. In fact, they represent the most common indication for frozen section evaluation of the liver and one of the most challenging problems encountered in the frozen section laboratory [1, 2]. The distinguishing features of benign ductal lesions and metastatic adenocarcinoma are summarized in Table 2.1.

Bile Duct Hamartoma (von Meyenberg Complex)

Bile duct hamartomas are well-circumscribed aggregates of biliary ductules embedded in a near-equal amount of hyalinized stroma (Fig. 2.2). These ductules are variably cystic and lined by flattened, or cuboidal, epithelium without cytologic atypia. Some bile duct hamartomas contain inspissated bile, but this finding is inconsistently present (Figs. 2.3 and 2.4). Multiple bile duct hamartomas are distributed along the portal tracts, reflecting their

Fig. 2.1 Multiple von Meyenberg complexes (bile duct hamartomas) are present under the capsule (arrows) and adjacent to portal tracts (asterisks).
<table>
<thead>
<tr>
<th>Feature</th>
<th>Bile duct hamartoma</th>
<th>Bile duct adenoma</th>
<th>Metastatic adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomic location</td>
<td>Subcapsular</td>
<td>Subcapsular</td>
<td>Variable</td>
</tr>
<tr>
<td>Size</td>
<td>&lt;1 cm</td>
<td>&lt;1 cm</td>
<td>Variable</td>
</tr>
<tr>
<td>Tumor centricity</td>
<td>Solitary or multiple</td>
<td>Solitary or multiple</td>
<td>Usually multiple</td>
</tr>
<tr>
<td>Quality of stroma</td>
<td>Densely collagenous</td>
<td>Cellular</td>
<td>Desmoplastic</td>
</tr>
<tr>
<td>Appearance</td>
<td>Circumscribed</td>
<td>Circumscribed</td>
<td>Ill-defined</td>
</tr>
<tr>
<td>Intrallesional portal tracts</td>
<td>Common</td>
<td>Common</td>
<td>Present at lesional edge</td>
</tr>
<tr>
<td>Organization of epithelium</td>
<td>Uniformly-spaced, variably diluted tubules</td>
<td>Uniformly-spaced, small round tubules</td>
<td>Irregular aggregates of variably sized glands</td>
</tr>
<tr>
<td>Luminal mucin</td>
<td>Absent</td>
<td>May be present</td>
<td>Present</td>
</tr>
<tr>
<td>Bile</td>
<td>May be present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Cytologic atypia</td>
<td>Absent</td>
<td>Minimal</td>
<td>Present</td>
</tr>
<tr>
<td>Mitoses</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>
Fig. 2.2 Bile duct hamartomas are well-circumscribed proliferations of dilated tubules associated with collagenous stroma.

Fig. 2.3 The tubules of a bile duct hamartoma are lined by cuboidal, or flattened, epithelial cells that lack cytologic atypia. The stroma is dense, paucicellular, and eosinophilic.
Fig. 2.4  Inspissated bile is present within the tubules of a bile duct hamartoma.

origin from malformations at the interface between limiting plate hepatocytes and the portal tracts (Fig. 2.5). Most bile duct hamartomas are readily distinguished from metastatic adenocarcinoma at low magnification. Bile duct hamartomas are round aggregates of ductules embedded in dense paucicellular collagen, whereas carcinoma deposits are ill-defined, expansile nodules composed of irregularly spaced glands and single cells enmeshed in desmoplastic stroma (Fig. 2.6). Carcinomatous glands are haphazardly arranged and angulated with highly atypical cells (Fig. 2.7).

Bile Duct Adenoma (Biliary Adenoma)
Bile duct adenomas are well-circumscribed proliferations of small tubules and stroma that are morphologically distinct from bile duct hamartomas (Fig. 2.8). They contain densely packed, proliferating ductules enmeshed within compact, cellular stroma (Fig. 2.9). These ductules are lined by plump, cuboidal epithelial cells with abundant faintly eosinophilic cytoplasm and display mild, if any, cytologic atypia (Fig. 2.10). Bile duct adenomas do not contain bile. Their recognition is quite problematic in some cases because the cellular stroma may simulate desmoplasia and the dense proliferation
**Fig. 2.5** Bile duct hamartomas occur along portal tracts and are often multiple.

**Fig. 2.6** Metastatic pancreatic ductal adenocarcinoma is an ill-defined, expansile nodule composed of irregularly spaced glands that are variably dilated and contain mucin.
Fig. 2.7 The infiltrative glands of metastatic pancreatic ductal adenocarcinoma are embedded in desmoplastic stroma. They are variable in size and shape and contain epithelial cells with severe cytologic atypia.

Fig. 2.8 Bile duct adenomas are well-circumscribed subcapsular proliferative lesions that contain numerous glands and stroma.
**Fig. 2.9** The glands of a bile duct adenoma are densely-packed and surrounded by cellular stroma.

**Fig. 2.10** Bile duct adenomas contain proliferating tubules lined by cuboidal cells with faintly eosinophilic cytoplasm and round nuclei. The stroma contains inflammatory cells, but is not desmoplastic.
of slightly atypical ducts may be mistaken for adenocarcinoma, particularly when the degree of cytologic atypia is enhanced by frozen section artifact (Fig. 2.11). This problem is compounded when bile duct adenomas contain mucin, since this finding has been considered a feature of adenocarcinoma (Fig. 2.12). Other criteria that aid the distinction between bile duct adenoma and adenocarcinoma include the well-circumscribed appearance of the former, the nature of its stroma, and presence of only mild cytologic atypia in these lesions.

**Inflammatory Changes in Preexisting Bile Ducts**

Patients with chronic ascending cholangitis develop persistent localized ductal dilatation in combination with hepatic scarring that may simulate the appearance of either primary or metastatic adenocarcinoma (Fig. 2.13). Carcinomas of the pancreatic head, distal bile duct, and/or ampulla may cause bile duct obstruction that produces ductular reactions in the liver that grow large enough to be identified during surgery. Large duct obstruction typically results in a bile ductular proliferation in the portal tracts with periportal edema and fibrosis. The ductules are variably dilated...
Fig. 2.12 Some bile duct adenomas contain mucin, which should not be interpreted as a diagnostic feature of metastatic adenocarcinoma.

Fig. 2.13 Chronic ascending cholangitis causes dilatation of bile ducts with periportal fibrosis. Localized organizing abscesses in the parenchyma form an ill-defined mass (arrow).
and show mild cytologic atypia with bile plugs and neutrophils, but they do not infiltrate adjacent parenchyma (Fig. 2.14). Long-standing obstruction may lead to extensive ductular proliferation in combination with inflammatory stroma that forms a mass. These ductules can simulate adenocarcinoma, especially when entrapped ductules appear angulated or compressed (Figs. 2.15 and 2.16).

**Fig. 2.14** Large duct obstruction causes bile ductular proliferation in portal tracts. The ducts are variable in size and some contain bile.

**Distinction Between Benign and Malignant Glandular Lesions in the Liver**

Metastatic adenocarcinomas of the pancreaticobiliary tree, stomach, esophagus, and gastroesophageal junction are close mimics of benign bile duct proliferations, especially bile duct adenomas. Helpful distinguishing features include the well-circumscribed nature of benign lesions and their near-complete lack of cytologic atypia (Table 2.1). Some hepatic nodules may not be classifiable as benign or malignant based upon frozen section analysis alone. In this situation, it is best to communicate concerns to the surgeon and defer final classification to permanent sections. An incorrect diagnosis of benignancy will result in continuation of surgery and resection of the primary tumor, whereas a diagnosis of metastatic
Fig. 2.15 Large duct obstruction may produce nodules of proliferating ductules associated with inflammation and loose inflammatory stroma.

Fig. 2.16 This patient had an obstructing pancreatic adenocarcinoma and a small hepatic nodule that was intraoperatively assessed during pancreatectomy. Angulated proliferating bile ducts in myxoid stroma mimic metastatic carcinoma. The frozen section interpretation was further hampered by knowledge that the patient had received neoadjuvant chemotherapy, which might be expected to induce fibrosis around metastatic carcinoma (same case as Fig. 2.15).
carcinoma will halt the procedure. We have, on rare occasion, asked the surgeon to delay a procedure to evaluate permanent sections and ensure a correct diagnosis. Immunohistochemical stains may also be used to facilitate the distinction between benign ductular proliferations and metastatic adenocarcinoma (Table 2.2) [3].

**Mucinous Cystadenoma (Hepatobiliary Cystadenoma)**

Mucinous cystadenoma of the liver was first described in association with pancreatic mucinous cystic neoplasms [4]. These tumors are more common among women and are unilocular, or multilocular, cysts with internal septations. Potentially life-threatening complications such as bleeding or perforation due to erosion into adjacent structures may occur [5]. Hepatic mucinous cystadenomas contain epithelial cells that resemble endocervical epithelium and show a spectrum of cytologic atypia, ranging from low- to high-grade dysplasia. Rare tumors precede invasive carcinoma. Mucinous cystadenomas in women typically contain dense “ovarian-type stroma,” whereas those that arise in men lack this feature (Fig. 2.17) [6]. Complete surgical removal is the treatment of choice in patients with symptomatic disease, radiographic features suspicious for malignancy, and high-grade epithelial cell dysplasia in material obtained by fine-needle aspiration. Intraoperative consultations may be obtained to assess the adequacy of surgical

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**Table 2.2** Immunohistochemical stains that distinguish bile duct adenoma from metastatic carcinoma.

<table>
<thead>
<tr>
<th>Immunohistochemical stain</th>
<th>Bile duct adenoma</th>
<th>Metastatic adenocarcinoma</th>
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<tbody>
<tr>
<td>p53</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>TAG-72</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>mCEA</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Mesothelin</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>BCL2</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>IMP3</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>CA125</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>AMACR</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>DPC4</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Ki67</td>
<td>&lt;35% staining of lesional nuclei</td>
<td>&gt;35% staining of lesional nuclei</td>
</tr>
</tbody>
</table>

Based on data from: Hornick et al. [3]; Tan et al. [15]
FROZEN SECTION LIBRARY: LIVER

resection or to make a primary diagnosis in those cases that have not been previously evaluated by cytology [7].

MALIGNANT BILE DUCT LESIONS OF THE LIVER

Cholangiocarcinomas are malignant epithelial tumors derived from the intrahepatic and/or extrahepatic bile ducts. These aggressive neoplasms are classified based upon their anatomic location. Those that develop within the liver are intrahepatic cholangiocarcinomas and are classified as central and peripheral types. Extrahepatic cholangiocarcinomas that arise at the bifurcation of right and left hepatic ducts are subclassified as proximal (hilar) cholangiocarcinomas, and distal cholangiocarcinomas occur distal to the bifurcation. Frozen section analysis of bile duct margin is commonly performed to guide the extent of resection for cholangiocarcinomas of the perihilar region, including both extrahepatic tumors that invade the liver (hilar cholangiocarcinoma or Klatskin tumor) and those that develop in the porta hepatis (intrahepatic central cholangiocarcinoma). Pathologists may be asked to perform frozen

Fig. 2.17 Mucinous cystadenomas contain epithelium that resembles endocervical cells overlying dense “ovarian-type stroma.” This tumor shows only low-grade dysplasia in lesional epithelium.
sections on bile duct margins present in hepatic resection specimens or separately submitted segments of extrahepatic bile duct distal to the tumor. Extrahepatic cholangiocarcinomas are discussed in detail in Chapter 6.

The reported risk factors for, and natural history of, intrahepatic cholangiocarcinoma mostly reflect the features of central tumors, which are much more common than peripheral lesions. Risk factors for development of cholangiocarcinoma include primary sclerosing cholangitis, hepatolithiasis, and parasitic infestation of the biliary tree by Opisthorchis viverrini and Clonorchis sinensis [8]. These liver flukes are endemic to parts of southeast and eastern Asia and account for the striking geographic distribution of intrahepatic cholangiocarcinoma [9]. The incidence of intrahepatic cholangiocarcinoma in the United States has been steadily increasing since the 1970s for unclear reasons, although it is notable that this rise parallels a concurrent increase in HCV infection rates [8]. Central cholangiocarcinoma is associated with a poor prognosis, and surgical resection is the only curative therapy since adjuvant therapy is of limited efficacy in disease management. Unfortunately, many patients have advanced tumors at clinical presentation or extensive hepatic fibrosis and insufficient hepatic reserves to undergo liver resection [10, 11]. Liver transplantation is a consideration in latter situation, provided patients have localized tumors, although approximately 50% of cholangiocarcinomas recur in the graft liver [11].

Central Cholangiocarcinoma

Central cholangiocarcinomas are ill-defined, gritty, gray–white masses that track along the central portal structures in proximity to the bile duct and or portal vein margins (Fig. 2.18). Most do not develop in patients with cirrhosis (Fig. 2.19). Often, the ligated bile duct, artery, and vein are not readily identifiable on hepatic resection specimens and, thus, must be clearly indicated by the surgeon. Frozen sections are routinely obtained on bile duct margins to confirm adequate tumor clearance and an absence of dysplasia, which may appear as a mucosal irregularity in the bile duct (Figs. 2.20 and 2.21). Sections of vascular and bile duct margins are generally taken en face, although one may argue that perpendicular sections provide a more accurate estimate of tumor clearance than en face margins. Indeed, submillimeter assessment of margins is probably an academic exercise. Patients with narrow (<1 mm), but negative, bile duct resection margins have recurrence rates similar to those of patients with microscopically positive margins (Fig. 2.22) [12, 13].
**Fig. 2.18** This central cholangiocarcinoma is a firm yellow–white tumor at the hilum. It is associated with bile staining of the liver due to obstruction.

**Fig. 2.19** This central cholangiocarcinoma encases the hilar structures. The background liver is essentially normal.
**Fig. 2.20** Polypoid lesions in the duct raise concern for neoplasia (*arrow*) and, prompt frozen section evaluation of the bile duct resection margin (same case as Fig. 2.19).

**Fig. 2.21** Frozen section of the polypoid lesion (same case as Fig. 2.20) reveals a complex papillary proliferation of dysplastic epithelium.
Outcomes are superior among patients with widely negative bile duct margins, such as those individuals with negative margins on both hepatic resection specimens and separately submitted bile duct segments [14].

Central and hilar cholangiocarcinomas contain overtly malignant, infiltrating glands and single cells enmeshed within desmoplastic stroma, similar to pancreatic ductal adenocarcinomas (Fig. 2.23). The diagnosis is generally straightforward when evaluating the primary tumor, but assessing bile duct margins is challenging. Invasive cholangiocarcinomas usually invade the bile duct wall, but spare the overlying mucosa (Fig. 2.24). Initial evaluation of bile duct margins is best achieved at low magnification, to appreciate the lobular architecture of benign periductal glands and distinguish them from infiltrating adenocarcinoma (Fig. 2.25). Periductal glands of the biliary mucosa show some degree of atypia due to either frozen section artifact or prior placement of a biliary stent (Figs. 2.26 and 2.27). Features suggesting a benign diagnosis include a rounded lobular architecture and mild atypia. This topic is discussed in further detail in Chapter 6.
**Fig. 2.23** Central cholangiocarcinomas contain irregularly spaced dilated glands associated with abundant desmoplastic stroma, similar to pancreatic ductal adenocarcinoma.

**Fig. 2.24** The epithelium of this bile duct is partially denuded and the wall of the duct is infiltrated by cholangiocarcinoma.
Fig. 2.25  Benign periductal glands are arranged in lobules and contain cells with round, regular, basally located nuclei and faintly eosinophilic cytoplasm.

Fig. 2.26  The epithelium of a previously stented bile duct is proliferative and displays a slightly papillary architecture.
Peripheral Cholangiocarcinoma

Peripheral cholangiocarcinomas are pale, relatively well-circumscribed, fleshy masses that occur distant from the hilum (Fig. 2.28). Most are resected via partial hepatectomy, so the most important margin is the hepatic parenchymal margin, which is inked and sectioned to demonstrate its relationship to the tumor (Figs. 2.28 and 2.29). Peripheral cholangiocarcinomas are morphologically distinct from central tumors, in that they are more cellular and composed of well-differentiated, neoplastic tubules with little intervening stroma (Fig. 2.30). The neoplastic cells are embedded in collagenous stroma that may be hyalinized (Fig. 2.31). Some tumors, particularly those in patients with underlying liver disease, have features of both adenocarcinoma and hepatocellular carcinoma (mixed hepatocellular/cholangiocarcinoma).

Fig. 2.27 This previously stented bile duct margin contains epithelial cells with mildly enlarged, basally oriented nuclei suggesting that the cytologic atypia is reactive (same case as Fig. 2.26).
Fig. 2.28 Most peripheral cholangiocarcinomas are yellow–white solitary tumors with a scalloped edge. The background liver is usually normal.

Fig. 2.29 This peripheral cholangiocarcinoma is 3 mm from the inked hepatic resection margin.
Fig. 2.30 Peripheral cholangiocarcinomas consist of well-differentiated tubules that may show minimal cytologic atypia.

Fig. 2.31 The glands of peripheral cholangiocarcinoma are embedded in hyalinized or collagenous stroma that is less cellular than the desmoplastic stroma typical of central cholangiocarcinomas.
References

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