The Many Faces (and Origins) of Adenocarcinoma in the Liver: Pattern Approach to Diagnosis on Small Tissue Samples

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ABSTRACT

Focal liver lesions can be solitary or multiple, solid or cystic, congenital or acquired, and range from cysts, hamartomas, hyperplastic nodules and inflammatory entities to tumors and tumor-like lesions. The background liver may be normal or diseased. An algorithmic approach based on pattern recognition and cell profiling is outlined for the diagnosis of focal liver lesions with glandular features in small tissue samples from fine needle aspiration and core needle biopsy. The morphological categories for lesions with glandular phenotypes are (i) glandular (ducts, glands and/or mucin) pattern, including biliary and papillary patterns, (ii) hepatocellular and epithelioid patterns; (iii) mixed epithelioid-glandular (hepatobiliary) pattern; (iv) predominant cell profiles; and (v) cystic pattern. Hepatobiliary entities should be segregated before considering nonhepatobiliary conditions. The main diagnostic issues are to distinguish cystic from solid lesions, primary from metastatic adenocarcinomas, poorly differentiated adenocarcinoma from poorly differentiated hepatocellular carcinoma; and to recognize rare glandular entities, mimics and pitfalls. Assessment of sample adequacy, key cytohistological features, and diagnostic utility of ancillary tests are addressed. Close clinicopathological correlation is mandatory before rendering a final definitive diagnosis.

Key Words: Adenocarcinoma, cholangiocarcinoma, core biopsy, fine needle aspiration cytology, liver, metastases
INTRODUCTION

The anatomical and functional complexity of the liver makes for a highly varied and challenging landscape of pathologies for clinical, radiological and pathological diagnosis. The liver consists of hepatobiliary and other cellular components, receives a dual blood supply, and has a monocyte-macrophage function. Focal liver lesions can be solitary or multiple, solid or cystic, congenital or acquired, and may range from cysts, hamartomas, hyperplastic nodules and inflammatory entities to tumors and tumor-like lesions.1, 2

Three clinicopathological scenarios come to mind when the commonplace adenocarcinoma is addressed in the liver.3, 4 First, the liver is a common depository for secondaries from virtually any part of the body. The majority are metastatic adenocarcinomas which have to be distinguished from primary intrahepatic cholangiocarcinoma (ICC). Second, the liver can undergo cirrhosis with subsequent development of hepatocellular carcinoma (HCC). The pseudoacinar pattern in HCCs and the rare combined hepatocellular-cholangiocarcinoma (CHCC-CC) can be confused with adenocarcinoma. Third, benign neoplastic and nonneoplastic biliary lesions can mimic adenocarcinoma.

Suffice it to say that the differential diagnoses abound when a carcinoma with putative glandular morphology is encountered. It is imperative for prognostic and therapeutic purposes that an accurate cytohistological diagnosis be rendered. This can prove extremely challenging on small tissue samples from fine needle aspiration biopsy (FNAB) and core needle biopsy (CNB).

This article aims to provide an algorithm for the pattern recognition and cell profiling of focal liver lesions with putative glandular morphology. Intrahepatic cholangiocarcinoma is taken as the reference tumor. Key cytohistological features, diagnostic utility of immunohistochemistry, and diagnostic pitfalls and challenges are addressed. Clinicopathological correlation is mandatory.

CLINICAL PERSPECTIVE

In clinical practice, the initial impression of a liver mass is to investigate for HCC or metastases rather than ICC, unless there is a positive supportive history such as primary sclerosing cholangitis (PSC), chronic biliary tract disease due to hepatobiliary lithiasis and liver flukes such as Clonorchis sinensis or Opisthorchis viverrini, or developmental cystic anomalies.1-5 Metastatic tumors with glandular morphology that are commonly encountered in the liver include those from colorectum, stomach, pancreas, extrahepatic biliary tract, breast, lung, prostate and female genital tract. The background liver may provide important clues. Metastases are rarely found in cirrhotic livers. Pre-existing benign cysts may call attention to themselves with adenocarcinomatous transformation.

Patients may be asymptomatic or present with advanced disease. They fall into several clinical settings:

- Investigation for symptomatology referable to the primary hepatic tumor or liver metastases such as obstructive jaundice, right hypochondrial pain, biliary sepsis or hepatomegaly
- Radiological staging or surveillance for recurrent or metastatic disease in known cancer patients
- Follow-up/surveillance of at-risk patients with pre-existing benign cystic condition or chronic hepatobiliary disorder
- Routine health screening revealing abnormal liver function test profiles, elevated serum tumor markers such as CA19-9 and carcinoembryonic antigen (CEA), or imaging abnormalities
**RADIOLOGICAL PERSPECTIVE**

Imaging features of ICC are categorized into mass-forming (MF), periductal infiltrating (PI), and intraductal growth (IG) types. The checklist that must be complied with includes exclusion of lesions in other sites, presence of peripheral duct dilatation, delayed enhancement of radiocontrast dye, and hilar lymphadenopathy. Cirrhosis should alert one to the possibility of an HCC or a CHCC-CC. The MF type with irregular borders is the commonest and typically shows arterial and portal venous phase rim-like enhancement and slow diffusion of contrast into the tumor leading to late central enhancement. The poorly enhanced center is due to necrosis and/or desmoplasia. Capsular retraction is characteristic. The PI and IG types usually present with obstructive jaundice. The PI type tends to simulate benign stricture with thickening and segmental enhancement of the bile ducts. The IG type due to polypoid or papillary growths is uncommon and frequently not detected on imaging as the lesions are usually small.

Liver is the most common intra-abdominal organ for metastases which are often multiple with variable appearances depending on the primary site. On ultrasound (US), most metastases appear as hypoechoic masses. On computed tomography (CT), the appearance is highly varied but usually hypodense. Hyperdense metastases can be seen in carcinoïd tumor and mucinous adenocarcinoma of colon when they calcify. Some metastases have nonspecific targetoid appearance. Cystic lesions appear typically anechoic on US, hypodense on CT, and hyperintense on T2-weighted magnetic resonance imaging (MRI). Mucinous cystic neoplasms (MCN) can resemble simple cysts and appear uniformly anechoic on US, with a barely perceptible wall and no internal echoes. Metastases can appear cystic, especially from mucinous adenocarcinomas or post-chemotherapy necrosis; and so can ICC. Imaging of biliary intraductal papillary neoplasm (IPN) may demonstrate dilated intra-/extrahepatic bile ducts with single or multiple polypoid masses in the biliary tree.

**PATHOLOGICAL PERSPECTIVE**

Cholangiocytes lining the small-caliber bile duct branches are small and flattened to cuboidal cells. The epithelium becomes columnar with occasional mucous cells in the larger ducts. It is speculated that ICCs found at these different anatomical locations portray a corresponding morphological topography. Those mixed hepatobiliary carcinomas (MHBC) portraying hybrid features straddling hepatocytes and cholangiocytes are likely attributed to hepatic stem and progenitor cells in the canals of Hering. This phenotypic diversity may be used to diagnostic advantage in distinguishing adenocarcinoma of biliary tract origin from other adenocarcinomas, apart from pancreatic adenocarcinoma since the biliary tract is regarded as an anatomical extension of the pancreas. Immunohistochemically, ICCs are characteristically CK7/CK19 positive and CK20 negative. However, those closer to the hilum may acquire additional CK20 positivity.

**DIAGNOSTIC ALGORITHM**

**Basic patterns and cell profiles**

The approach to the evaluation of small tissue samples begins with the consideration of whether the specimen is from a solid or cystic lesion to enable assessment of adequacy and representativeness. The morphological categories for lesions with glandular phenotypes are (i) glandular (ducts, glands and/or mucin) pattern, including biliary and papillary patterns; (ii) hepatocellular and epithelioid patterns; (iii) mixed epithelioid-glandular (hepatobiliary)
pattern; (iv) predominant cell profiles; and (v) cystic pattern (Table 1).

Hepatobiliary entities should be segregated before considering nonhepatobiliary lesions. Differential diagnoses and diagnostic pitfalls are discussed.

(i) Glandular (ducts, glands and/or mucin) pattern, including biliary and papillary patterns

The most important primary adenocarcinoma in the liver is ICC although metastases are by far more common. Mixed hepatobiliary carcinomas, and MCN and biliary IPN with their associated invasive carcinomas, are addressed in sections (iii) and (v), respectively.

The typical biliary epithelium comprises monolayered honeycomb sheets of cuboidal/columnar epithelium. The equidistant, round to oval nuclei are bland with no nucleolus normally. The cell borders are indistinct. Reactive and atypical changes are indicated by the increasing presence of prominent nucleoli, nuclear overlapping, and abrupt nuclear enlargement, leading to a drunken honeycomb appearance. Nuclear pleomorphism with high nuclear-to-cytoplasmic ratio, nuclear membrane irregularities, hyperchromasia, and mitoses appear with increasingly higher grades of biliary intraepithelial neoplasia (BilIN), progressing to in-situ malignant transformation (Fig. 1).

Intrahepatic cholangiocarcinomas are notoriously heterogeneous even over a small area (Table 2).

Classic ICCs can be graded into well, moderately and poorly differentiated adenocarcinomas (Fig. 2 and 3). Three smear patterns can be recognized on low power scanning, namely: (i) Scanty cell type: This “background rich-tumor cell poor” pattern typically yields sparse, easily overlooked tumor cells entrapped in desmoplastic stroma; (ii) Ductular proliferation type: This “background moderate-tumor cell modest” pattern is a cue for ICC comprising larger tumor cells admixed with plentiful bland, small ductular cells reflecting ductular reaction to chronic hepatic injury; and (iii) Usual adenocarcinoma type: This “background poor-tumor cell rich” pattern comprising larger sheets of pleomorphic glandular cells exhibiting cribriform, palisading and ductal appearances, is not characteristic of ICC and requires other biliary identifiers.

Variations and variants of ICC pose challenges to diagnosis on small samples (Fig. 4). Flat sheets, branching structures with peripheral nuclear palisading, papillary structures with fibrovascular cores, 3-dimensional acinar spaces, intracytoplasmic mucin secretion (signet ring cells), and adenosquamous components can be encountered solely or in combination. This striking phenotypic diversity is exemplified even in tissue cores. Histologically, the malignant cells may be arranged in irregular tubular, tubulocystic, nested glandular or papillary configurations, and cordlike, with a predilection for subepithelial zones beneath pre-existing bile ducts. “Adenofibromatous” or “pseudoangiomatoid” arrangement reminiscent of von Meyenburg complexes is unique. Transition from relatively bland-looking bile duct cells to dysplastic (BilIN)/malignant cells (carcinoma-in-situ) is a diagnostic clue. Papillary pattern is more likely to indicate metastases or ICC rather than the rare biliary IPN.

It is virtually impossible to distinguish ICC from metastatic pancreaticobiliary adenocarcinomas in the absence of intrahepatic BilIN or carcinoma-in-situ as they have similar immunoprofiling (Fig. 5) (Table 3). Adenocarcinomas with “dirty” necrosis are likely to be colorectal metastases but not exclusively (Fig. 6). Colorectal metastases are notoriously known for their propensity to colonize the existing intrahepatic biliary tree resulting in an intraductal
Table 1: Algorithmic approach to the morphological diagnosis of adenocarcinomas occurring in the liver.

<table>
<thead>
<tr>
<th>Patterns and predominant cell profiles</th>
<th>Diagnostic considerations</th>
<th>Diagnostic pitfalls and differential diagnosis</th>
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<tbody>
<tr>
<td></td>
<td><strong>Hepatobiliary lesions</strong></td>
<td><strong>Nonhepatobiliary lesions</strong></td>
</tr>
</tbody>
</table>
| (i) Glandular (ducts, glands ± mucin), including biliary and papillary patterns | • Intrahepatic cholangiocarcinoma (ICC), and variants  
• Mixed hepatobiliary carcinoma  
• Mucinous cystic neoplasm (MCN) with associated invasive carcinoma  
• Biliary intraductal papillary neoplasm (IPN) with associated invasive carcinoma | • Metastases from adenocarcinomas in general  
• Metastases from pancreaticobiliary carcinomas  
• Metastases from papillary carcinomas (pancreas, lung, ovary)  
• AFP-producing (nonhepatoid) adenocarcinoma, metastatic  
• Metastases from acinar adenocarcinomas (prostate, pancreas, salivary glands)  
• Neuroendocrine tumor/carcinoma | • Benign conditions such as bile duct hamartoma, bile duct adenoma (peribiliary gland hamartoma), biliary adenofibroma and serous cystadenoma  
• MCN and biliary IPN (malignant focus not sampled)  
• Pseudopapillary appearance mistaken for papillary pattern  
• Papillary pattern overlooked for poorly differentiated tumor  
• Ductular reaction, e.g. in scar of focal nodular hyperplasia  
• Inflammatory pseudotumor with reactive biliary atypia  
• Gastrointestinal contaminants (EUS-FNA)  
• Epithelioid hemangioendothelioma |
| (ii) Hepatocellular and epithelioid patterns | • Mixed hepatobiliary carcinoma  
• ICC, and variants with hepatoid features | • Extrahepatic hepatoid carcinoma, metastatic  
• Metastases from epithelioid variants of sarcomas | • Hepatocellular carcinoma (HCC) with CK19 positivity  
• HCC with pseudoacinar pattern |
| (iii) Mixed epithelioid-glandular (hepatobiliary) pattern | • Mixed hepatobiliary carcinoma | | • Collision tumors (HCC and ICC)  
• HCC (predominant hepatocellular component)  
• Adenocarcinoma (predominant glandular component)  
• Poorly differentiated carcinoma (predominant transitional component) |
Table 1 Algorithmic approach to the morphological diagnosis of adenocarcinomas occurring in the liver. (con.)

<table>
<thead>
<tr>
<th>Patterns and predominant cell profiles</th>
<th>Diagnostic considerations</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Hepatobiliary lesions</td>
<td>Nonhepatobiliary lesions</td>
</tr>
<tr>
<td>(iv) Cell type: Squamous cell</td>
<td>• ICC, adenosquamous type</td>
<td>• Metastases from adenosquamous carcinomas (pancreas, extrahepatic biliary tract, cervix, lungs)</td>
</tr>
<tr>
<td>Small/intermediate</td>
<td>• ICC with small cell features</td>
<td>• Neuroendocrine tumor/carcinoma (small cell type)</td>
</tr>
<tr>
<td>Large cell</td>
<td>• ICC, high grade</td>
<td>• Neuroendocrine carcinoma (large cell type)</td>
</tr>
<tr>
<td>Polygonal</td>
<td>• ICC with hepatoid features</td>
<td>• Extrahepatic hepatoid carcinoma, metastatic</td>
</tr>
<tr>
<td>Clear cell</td>
<td>• ICC with clear cell features; foamy cell variant</td>
<td>• Renal cell carcinoma, metastatic</td>
</tr>
<tr>
<td>Oncocytic cell</td>
<td>• ICC with hepatoid features</td>
<td>• Extrahepatic hepatoid carcinoma, metastatic</td>
</tr>
<tr>
<td>Transitional cell</td>
<td>• Mixed hepatobiliary carcinoma</td>
<td>• Poorly differentiated carcinoma, metastatic</td>
</tr>
<tr>
<td>Pleomorphic cell</td>
<td>• ICC, high grade</td>
<td>• Poorly differentiated carcinoma, metastatic</td>
</tr>
<tr>
<td>Spindle cell</td>
<td>• ICC, sarcomatoid type</td>
<td>• Sarcomatoid carcinoma, metastatic</td>
</tr>
<tr>
<td>Giant cell</td>
<td>• ICC, sarcomatoid type</td>
<td>• Sarcomatoid carcinoma, metastatic</td>
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<tr>
<td>Inflammatory cell</td>
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<tr>
<td>(v) Cystic pattern</td>
<td>• MCN with associated invasive carcinoma</td>
<td>• Cystic metastases (ovary and pancreas)</td>
</tr>
<tr>
<td></td>
<td>• Biliary IPN with associated invasive carcinoma</td>
<td>• Neuroendocrine tumors/carcinoma (with cavitating necrosis)</td>
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<tr>
<td></td>
<td>• Cystic ICC</td>
<td></td>
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<tr>
<td></td>
<td>• Carcinoma associated with developmental cysts</td>
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</table>
### Table 2: Key morphological and immunohistochemical features of intrahepatic cholangiocarcinoma (ICC).

<table>
<thead>
<tr>
<th>Immunohistochemistry</th>
<th>Histology</th>
<th>Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK7+/CK20- (CK14/CK20+ for tumors nearer hilum)</td>
<td>Continuous unbroken cores, usually obtained</td>
<td>Cellularity: Scant, modest to plentiful malignant cells</td>
</tr>
<tr>
<td></td>
<td>Heterogeneity in cell pattern and cell arrangement (phenotypic diversity)</td>
<td>Cell arrangement: Tubular, tubuloacinar, nests of glandular cells</td>
</tr>
<tr>
<td></td>
<td>Epithelial membrane antigen (EMA)</td>
<td>Large-sized malignant cells</td>
</tr>
<tr>
<td></td>
<td>Carcinoembryonic antigen (CEA)</td>
<td>Malignant cells with cytological atypia</td>
</tr>
<tr>
<td></td>
<td>MOC-31</td>
<td>Heterogeneity in cell pattern and cell appearance (phenotypic diversity)</td>
</tr>
<tr>
<td></td>
<td>CK19</td>
<td>Transition from bland bile duct cells to dysplastic/malignant cells is a diagnostic clue</td>
</tr>
<tr>
<td></td>
<td>CK7+ / CK 20- (CK7+/CK20+ for tumors nearer hilum)</td>
<td>Biliary intraepithelial neoplasia (BilIN) and other aberrant bile ducts can be seen in hepatocytes or canalicular cell plates at interface</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intraductal papillary neoplasms of the bile ducts (IPMN) can be seen in hepatocytes or canalicular cell plates at interface</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bile duct or vascular dilatation at portal tract interface regions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>One or two distinctively recognizable components may be identified on small tissue forms; or they may be camouflaged under hybrid forms, both morphologically and immunohistochemically</td>
</tr>
</tbody>
</table>

**Adapted from Table 2.9 in Wee et al, Cytohistology of focal liver lesions, Cambridge University Press, 2014.**
### Table 3 Common metastatic adenocarcinomas to the liver and their morphological and immunohistochemical characteristics.

<table>
<thead>
<tr>
<th>Tumor type and origin</th>
<th>Cytology</th>
<th>Histology</th>
<th>Immunohistochemistry</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma, colorectum</td>
<td>• Columnar epithelial cells in cohesive clusters +/- lumens • Broken end-on strips • Cigar-shaped, palisaded nuclei with prominent nucleoli • Intra-/extracytoplasmic mucin • Dirty necrosis</td>
<td>• Large well-formed malignant glands with necrotic lumen (“dirty necrosis”) • Desmoplasia • Intra-/extracytoplasmic mucin</td>
<td>CK7 -, CK20 + • CDX2 • Villin • Carcinoembryonic antigen (CEA) • MOC-31 • MUC1 • MUC2 • MUC3</td>
<td>One of the most common sites of origin for metastatic adenocarcinoma to the liver • Generally well- to moderately differentiated adenocarcinoma • Tumor diathesis is key clue</td>
</tr>
<tr>
<td>Adenocarcinoma, stomach</td>
<td>• Columnar epithelial cells in cohesive clusters +/- lumens • Broken end-on strips with basal palisaded oval nuclei • Dissociated signet-ring or plasmacytoid cells with eccentric hyperchromatic nuclei • Intra-/extracytoplasmic mucin • Necrosis</td>
<td>• Well to poorly differentiated tubules • Signet ring or plasmacytoid cells • Intra-/extracytoplasmic mucin • Desmoplasia</td>
<td>CK7 +/-, CK20 +/- No predominant pattern • CEA • Epithelial membrane antigen (EMA) • MUC1 • MUC2 • MUC5AC</td>
<td>Subtypes: Adenocarcinoma (intestinal, diffuse); papillary, tubular, mucinous and signet ring adenocarcinoma; and adenosquamous, small cell, and undifferentiated carcinoma</td>
</tr>
<tr>
<td>Adenocarcinoma, pancreas</td>
<td>• Cellularity varies with degree of desmoplasia • Sheets, cohesive clusters to discohesive glandular cells of variable pleomorphism • Stromal fragments with entrapped tumor cells • Intra-/extracytoplasmic mucin • Squamous elements +/-</td>
<td>• Well- to poorly differentiated adenocarcinoma • Architecturally well-formed glands but marked cytological atypia • Periductal concentric desmoplasia • Intra-/extracytoplasmic mucin • Squamous component +/-</td>
<td>CK7 +/-, CK20 +/- • CK5/6 +/- • EMA • CEA • CA19-9 • CDX2 • Villin • MUC1 • p53</td>
<td>Usually indistinguishable from biliary tract carcinomas, requiring clinical correlation • Well-differentiated adenocarcinoma can mimic benign glands • Desmoplasia favor pancreaticobiliary primary • Variants: Adenosquamous, oncocytic, clear cell, hepatoid, signet ring, basaloid, intestinal type, mucinous, squamous cell, and anaplastic (pleomorphic, sarcomatoid or undifferentiated) carcinoma</td>
</tr>
</tbody>
</table>
### Table 3: Common metastatic adenocarcinomas to the liver and their morphological and immunohistochemical characteristics. (con.)

<table>
<thead>
<tr>
<th>Tumor type and origin</th>
<th>Cytology</th>
<th>Histology</th>
<th>Immunohistochemistry</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Adenocarcinoma, gallbladder and extrahepatic biliary tract | - Cellularity varies with degree of desmoplasia  
- Monolayered sheets and clusters to discohesive glandular cells of variable pleomorphism  
- Stromal fragments with entrapped tumor cells  
- Heterogeneity of cells within drunken honeycomb sheets, nuclear overlapping, increased nuclear-to-cytoplasmic ratio and nucleolar prominence  
- Intra-/extracytoplasmic mucin  
- Squamous elements +/- | - Well- to poorly differentiated adenocarcinoma  
- Architecturally well-formed glands but poorly differentiated at cytological level  
- Heterogeneity of cells within same gland, increased nuclear-to-cytoplasmic ratio and nucleolar prominence  
- Periductal concentric desmoplasia  
- Intra-/extracytoplasmic mucin  
- Squamous component +/- | - CK7 +/-, CK20 +/-  
- EMA  
- CEA  
- p53 | - Usually indistinguishable from pancreatic adenocarcinomas, requiring clinical correlation  
- Metastatic well-differentiated adenocarcinoma can mimic benign glands  
- Desmoplasia favor pancreaticobiliary primary  
- Variants: Papillary, adenosquamous, clear cell, signet ring, lymphoepithelioma-like, and anaplastic (pleomorphic, sarcomatoid, undifferentiated) carcinoma; and carcinoma with neuroendocrine features |
| Adenocarcinoma, lung | - Polygonal to columnar epithelial cells arranged in monolayered sheets, 3D clusters or singly  
- Lumens may be discernible in some clusters  
- Variable nuclear atypia, ranging from bland to obviously malignant  
- Cytoplasm is delicate, fragile and frequently vacuolated  
- Intra-/extracytoplasmic mucin | - Spectrum of glandular differentiation ranging from well-differentiated tubular pattern to poorly differentiated type, bordering on undifferentiated large cell carcinoma  
- Intra-/extracytoplasmic mucin | - CK7 +, CK20 -  
- Thyroid transcription factor -1 (TTF-1) (nucleus)  
- EMA  
- CEA  
- CDX2 – (+ for mucinous type) | - Liver metastases are usually suspected clinically  
- Variants: Papillary, mucinous, and adenosquamous carcinoma |
Table 3: Common metastatic adenocarcinomas to the liver and their morphological and immunohistochemical characteristics. (con.)

<table>
<thead>
<tr>
<th>Tumor type and origin</th>
<th>Cytology</th>
<th>Histology</th>
<th>Immunohistochemistry</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal carcinoma, breast</td>
<td>• Monomorphous cell population</td>
<td>• Monomorphous appearance</td>
<td>CK7 +, CK20 -</td>
<td>In most cases, liver metastases are symptomatic</td>
</tr>
<tr>
<td></td>
<td>• Flat, angulated groups</td>
<td>• Sheets, small nests, cords or individual cells</td>
<td>• Estrogen (ES) and progesterone (PG) receptors +/- (not specific)</td>
<td>Occult primary is rare</td>
</tr>
<tr>
<td></td>
<td>• Single flame or cone-shaped cells</td>
<td>• Tubules +/-</td>
<td>• GATA3</td>
<td>Variants: Papillary, mucinous, medullary, metaplastic, and squamous cell carcinoma</td>
</tr>
<tr>
<td></td>
<td>• Target cells (cells with intracytoplasmic lumen)</td>
<td>• Tumor cells exhibit variable nuclear pleomorphism, increased nuclearto-cytoplasmic ratio and prominent nucleoli</td>
<td>• Gross cystic disease protein-15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Cell-in-cell arrangement</td>
<td>• Desmoplasia</td>
<td>• Mammaglobin</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• EMA</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>• CEA</td>
<td></td>
</tr>
<tr>
<td>Gyneocological tract carcinomas:</td>
<td>• Rosettes or tight clusters of columnar cells with nuclear palisading</td>
<td>• Conventional adenocarcinoma</td>
<td>CK7 +, CK20 -</td>
<td>Metastatic lesions may represent lymphovascular or peritoneal spread.</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>• Dirty necrosis</td>
<td>• Tubular lumens show dirty necrosis</td>
<td>• ES and PG receptors +/-</td>
<td>Variants: Papillary, adenosquamous and papillary serous carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Papillary (villoglandular) foci</td>
<td>• HER2/neu (c-erbB2)</td>
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<tr>
<td></td>
<td></td>
<td>• Squamous differentiation +/-</td>
<td>• Vimentin</td>
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<td></td>
<td></td>
<td>• CEA</td>
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<td></td>
<td>• CA-125</td>
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<td></td>
<td></td>
<td>• MUC1</td>
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<tr>
<td>Ovarian mucinous carcinoma</td>
<td>• Columnar epithelial cells with nuclear palisading and mucin secretion</td>
<td>• Adenocarcinoma with mucin secretion</td>
<td>CK7 +, CK20 +</td>
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<td></td>
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<td></td>
<td>• EMA</td>
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<td>• CEA</td>
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<td></td>
<td></td>
<td></td>
<td>• CDX2</td>
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<tr>
<td>Ovarian carcinoma, serous and endometrioid</td>
<td>• Columnar epithelial cells with nuclear palisading</td>
<td>• Adenocarcinoma</td>
<td>CK7 +, CK20 -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Papillary forms with high-grade cytological atypia</td>
<td>• Papillary carcinoma, high grade</td>
<td>• WT1</td>
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<tr>
<td></td>
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<td>• CA-125</td>
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<td>• EMA</td>
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<td></td>
<td></td>
<td>• Vimentin</td>
<td></td>
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<tr>
<td>Prostatic acinar adenocarcinoma</td>
<td>• Rosette-like groups of polygonal cells with pale/vacuolated cytoplasm,</td>
<td>• Variety of appearances corresponding to Gleeson score</td>
<td>CK7-, CK20 -</td>
<td>Differential diagnoses include acinar carcinoma of pancreas and well-differentiated HCC with pseudoacinar pattern</td>
</tr>
<tr>
<td></td>
<td>central nuclei and distinct nucleolus.</td>
<td>• Small to medium-sized glands to dissociated cells</td>
<td>• Prostate-specific antigen (PSA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Pale cytoplasm, increased nuclearto-cytoplasmic ratio and prominent nucleolus</td>
<td>• Prostatic acid phosphatase (PAP)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• EMA</td>
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</tr>
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</table>

Adapted from Table 7.2 from Wee et al. Cytohistology of focal liver lesions, Cambridge University Press, 2014.
growth pattern simulating ICC, intraductal type. CK20 versus CK7/CK19 immunoprofiling is helpful. However, the distinction may not be made in cytology material if one is not mindful of such as an occurrence. In the presence of signet ring cell adenocarcinoma, one must first and foremost exclude metastasis from a primary gastric carcinoma. Metastases from ductal carcinoma of breast should come to mind if the tumor cells appear rather monomorphic comprising dissociated plasmacytoid cells (Fig. 7). Rarely, an extrahepatic α-fetoprotein-producing adenocarcinoma, for example, from the stomach, may metastasize to the liver.22

Acinar pattern should be distinguished from pseudoacinar structures which are rosette-like arrangements of tumor cells about a space without true luminal brush border or mucin secretion. Common sources of acinar tumors include prostate and pancreas (Fig. 8). Neuroendocrine tumors and HCC often exhibit pseudoacinar pattern (Fig. 9).23-24 The absence of stromal matrix, and in its place the presence of (blood-filled) sinusoidal spaces between pseudoacini, confirms the trabecular-sinusoidal pattern of HCC.

Benign mimics of adenocarcinomas are treacherous. Bile duct hamartoma (von Meyenburg complexes), bile duct adenoma (peribiliary gland hamartoma) and biliary adenofibroma can mimic well-differentiated ICCs.2 The former two conditions are small subcapsular tumorlike nodules detected incidentally during laparoscopy/laparotomy staging for other gastrointestinal malignancies and sent for intraoperative frozen section consultation. Reactive atypia in biliary epithelium and peribiliary glands is often associated with cholangitis, calculous disease, stenting, PSC, and fluke infestation; it has to be distinguished from BilIN and malignant glands. Ductular reaction represents the restoration process at the damaged hepatocellular-stromal interface.21 Ductular epithelial clusters occurring as small dark overlapping nuclei in curved double-cell cords or tramline configurations are often seen in the central scar of focal nodular hyperplasia, and in association with cirrhosis, and large regenerative and dysplastic nodules. They should not be mistaken for cholangiolocellular carcinoma or small cell-type of adenocarcinoma.10, 11 The threshold for diagnosing malignancy should be raised in the event of finding biliary or peribiliary glandular atypia (reactive or degenerative) in the presence of persistent inflammation, such as in inflammatory pseudotumors;25, 26 Degenerative swelling of cells and nuclei coupled with nuclear pyknosis and vacuolated cytoplasm should not be mistaken for pleomorphism with increased nuclear-to-cytoplasmic ratio and hyperchromasia. Gastrointestinal contamination of endoscopic ultrasound (EUS)-FNA material is also a known pitfall. A rare mimic of (signet ring cell) adenocarcinoma is epithelioid hemangioendothelioma. The individual endothelial tumor cells with intracytoplasmic spaces and eccentric nuclei may or may not contain erythrocytes.27 In cases where the adenocarcinoma is poorly differentiated, distinction from a poorly differentiated HCC or metastases can be challenging. Ancillary tests may or may not be helpful. Correlation with clinical and radiological findings is imperative.

In summary, some features that favor ICCs over metastatic adenocarcinomas apart from those of pancreaticobiliary origin are heterogeneity, insinuative invasion at host-tumor interface, accompanying ductular reaction, pseudoangiomatoid appearance, entrapped portal tract structures, and biliary in-situ change. Metastases generally tend to be more monomorphic.4

(ii) Hepatocellular and epithelioid patterns

Areas with hepatoid/epithelioid features that
stain positively for CK7/CK19 are not uncommon in pure ICCs. Another consideration is MHBC [see section (iii)]. A nondescript epithelioid pattern is often encountered in poorly differentiated ICCs (Fig. 3). In the event that one can only arrive at a diagnosis of a poorly differentiated carcinoma, not otherwise specified, the cytohistology report may include the statement that “features are not specific for any primary site; correlate with clinical and radiological findings”.

(iii) Mixed epithelioid-glandular (hepatobiliary) pattern

An ambivalent glandular/epithelioid pattern may represent MHBC. In the classic subtype, combined hepatocellular-cholangiocarcinoma (CHCC-CC), one must demonstrate the cytomorphic and immunohistochemical presence of malignant hepatocytes, adenocarcinoma, and hybrid (transitional) elements (Fig. 10). Sampling error is a distinct pitfall in small tissue samples with the likelihood of the tumor being labeled as HCC, adenocarcinoma or a poorly differentiated carcinoma.

(iv) Predominant cell profiles

The variants of ICCs may display polygonal, oncocytic, small cell, large cell, clear cell, pleomorphic cell, spindle cell or giant cell features. Squamous component may be part of an adenosquamous carcinoma commonly encountered in the pancreaticobiliary tract. The differential diagnoses are listed in Table 1.

(v) Cystic pattern

Fine needle aspiration of neoplastic cysts such as MCN and biliary IPN with or without associated malignant transformation, may not yield totally diagnostic material; likewise for malignant foci developing within cystic anomalies. Targeted placement of the biopsy needle into suspicious areas under radiological guidance may enhance the diagnostic yield (Fig. 11). A cystic appearance can be encountered in mucin-producing ICCs or metastases. Cystically dilated bile ducts can result from obstructive ICCs. Extensive cavitating necrosis from post-therapy effects or otherwise can result in cystic imaging appearances.

Diagnostic utility of immunohistochemistry

Adenocarcinomas present the most difficulty in establishing their site of origin. Metastatic adenocarcinoma is one of the most common tumors encountered in the liver with the majority originating in the gastrointestinal tract, pancreaticobiliary tract, lung, breast and gynecological tract (Table 3). In these times where therapeutic protocols are standardized based on the site of tumor origin and their molecular profiles, it is noted that a diagnosis of adenocarcinoma may not suffice for clinical management. In such cases, clinical information, imaging studies and finally judicious use of immunohistochemical stains can help refine the histogenesis and pinpoint the likely site of origin.

The aim of immunohistochemistry in the evaluation of focal liver lesions with glandular features is two-fold, namely (i) to distinguish primary ICC from metastatic adenocarcinomas, and (ii) to define the various subsets of carcinomas in the liver that can be possible differential diagnosis. The initial immunohistochemical segregation of adenocarcinomas is based on the coordinate expression of CK7 and CK20, listed as follows:

- **CK7 + / CK20 + pattern:** Transitional cell carcinoma, pancreatic adenocarcinoma, biliary ( hilar) adenocarcinoma, and ovarian mucinous carcinoma
- **CK7 + / CK20 – pattern:** Lung adenocar-
cinoma, pancreaticobiliary adenocarcinoma, ductal and lobular breast carcinoma, ovarian carcinoma of serous and endometrioid type, endometrial carcinoma, and (mesothelioma)

- **CK7 - / CK20 + pattern:** Colorectal adenocarcinoma
- **CK7 - / CK20 – pattern:** HCC, renal cell carcinoma, prostatic adenocarcinoma, lung squamous cell carcinoma, and lung small cell carcinoma
- **No predominant pattern:** Gastric adenocarcinoma

Immunohistochemical stains commonly employed include polyclonal carcinoembryonic antigen (pCEA), CD10, MOC-31, and the cytokeratins.\(^{40-43}\) Polyclonal CEA highlights bile canaliculi but not hepatocytes in normal tissue. Biliary epithelial cells show diffuse cytoplasmic and brush border staining. In high-grade HCC, there is loss of canalicular expression but acquisition of cytoplasmic staining in about 50% cases. This staining pattern poses a challenge in distinguishing HCC from metastatic adenocarcinomas. It is, therefore, important that this stain is utilized keeping in mind the morphological differential diagnosis. CD10 shows similar staining patterns.

MOC-31, also known as epithelial specific antigen, is present in the cytoplasm as well as on cell surface in almost all epithelia, except in most squamous epithelia, hepatocytes, renal proximal tubular cells and gastric parietal cells. It is almost always (80% - 100%) expressed in ICC and metastatic adenocarcinoma from a variety of sites, including but not limited to carcinomas of the pancreas, breast, lung and stomach. This antibody, hence, cannot distinguish ICCs from metastatic adenocarcinomas to the liver. It is, however, either not expressed or is only weakly and focally expressed in HCC.\(^{44,45}\)

Of the cytokeratins, mature hepatocytes stain with CK8 and 18 and CAM5.2 but not with CK7, 19 or 20 or the commonly used CK cocktail, AE1/AE3. Intermediate hepatocytes may show lighter staining with CK7 but not with CK19. CK7 and 19 highlight biliary epithelium (Fig. 12). Focal CK7 positivity can be seen in poorly differentiated HCCs. Hence, the commonly used keratin antibodies (AE1/AE3 and CK7) can be expressed in both HCC and adenocarcinoma, limiting their value. CK7 and 20 are more helpful in determining the primary site once the diagnosis of adenocarcinoma has been established (Fig. 13).

Cytokeratin 19 is expressed in bile duct epithelium but not generally expressed in hepatocytes. CK19 is expressed in 85% to 100% of ICCs, whereas most HCCs are either negative or show patchy staining, with the exception of fibrolamellar HCC. Mixed hepatobiliary carcinomas may contain a variable proportion of hepatocyte progenitor cells. In such instances, a bi-directional differentiation of neoplastic progenitor cell populations is noted with expression of CK19 as well as CK8 and 18.

**SUMMARY**

The diagnostic approach is first to evaluate the tissue samples blinded to the clinical and radiological data. Only after a cytomorphological impression is established should one review the available data, before deciding on what special stains and panel of immunohistochemical stains to perform as there is usually limited material. The final diagnosis is based on close clinicopathological correlation. Preparation of cell blocks is very useful for ancillary tests.
Figure 1  Biliary epithelial changes; FNAB and core biopsy of liver.

(A) Biliary intraepithelial neoplasia (BillIN): Large flat sheet of biliary epithelium begins to lose the orderly honeycomb appearance due to variation in nuclear size and shape and some nuclear overlapping. The nuclei have coarse chromatin and small nucleolus. Papanicolaou, x200.

(B) Biliary adenocarcinoma: Sheets of malignant ductal epithelium show marked disorderly growth with crowding and nuclear overlapping. The variably-shaped nuclei display nuclear membrane irregularities with folds, chromatin clumping, increased nuclear-to-cytoplasmic ratio, and occasional nucleolus. The cytoplasm is pale and vacuolated. Papanicolaou, x200.

(C) BillIN and adenocarcinoma: A resident bile duct (right) exhibits low- to intermediate grade intraepithelial neoplasia with abrupt nuclear atypia and stratification. Malignant glands invade the vicinity which shows desmoplasia and lymphovascular invasion. Hematoxylin & eosin, x100.
**Figure 2** Intrahepatic cholangiocarcinoma, well-differentiated; core biopsy of liver with imprint cytology.

(A) Cluster of columnar epithelium with basal nuclei from a malignant gland. May-Grünwald-Giemsa, x400.

(B) Clusters and strips of malignant columnar glandular epithelium with nuclear palisading. The nuclei exhibit pleomorphism, coarse chromatin and small nucleolus. Nonneoplastic hepatocytes are seen at the right lower quadrant. Papanicolaou, x200.

(C) Well-differentiated malignant glands lined by columnar epithelium amid desmoplastic stroma. Hematoxylin & eosin, x100.

(D) CK7 highlights the malignant glands. Immunostain, x100.
**Figure 3** Intrahepatic cholangiocarcinoma, poorly differentiated; core biopsy of liver.

(A) There are sinuous trabeculae of tumor cells exhibiting eosinophilic cytoplasm and round hyperchromatic nuclei. Occasional cytoplasmic vacuoles are discernible. Tumor necrosis is present. Hematoxylin & eosin, x100.

(B) Left panel. Tumor cells contain intracytoplasmic mucin vacuoles. PAS-diastase, x 400.
Right panel. CK19 highlights the tumor cells. Immunostain, x200.

**Figure 4** Intrahepatic cholangiocarcinoma, variants; core biopsies of liver.

(A) Papillary carcinoma shows fibrovascular cores lined by well-differentiated malignant columnar epithelium. Hematoxylin & eosin, x100.
(B) Signet ring adenocarcinoma cells show eccentric hyperchromatic nuclei and intracytoplasmic mucin vacuoles accompanied by desmoplasia. Hematoxylin & eosin, x200.

Inset: CK7 highlights tumor cells. Immunostain, x 400.

(C) Adenofibromatous variant with pseudoangiomatoid pattern of growth shows intercommunicating spaces lined by largely flattened epithelium and separated by hyalinized collagenous stroma invaded by small malignant glands. Hematoxylin & eosin, x100.

Figure 5 Adenocarcinomas from pancreas; FNAB and core biopsy of liver metastases.

(A) Sheet of well-differentiated adenocarcinoma cells shows loss of monolayered honeycomb appearance with crowding and nuclear overlapping. Small nucleoli are discernible. Papanicolaou, x200.

(B) Mucinous pools in which float small groups or dissociated malignant glandular cells with enlarged nuclei, high nuclear-to-cytoplasmic ratio and intracytoplasmic mucin vacuoles. May-Grünwald-Giemsa, x200.

(C) Corresponding mucinous adenocarcinoma. Hematoxylin & eosin, x100.
Figure 6  Adenocarcinoma from colorectum; FNAB of liver metastases.

Left panel. Tightly cohesive clusters of malignant glands amid striking “dirty necrosis” (tumor diathesis) in the background. Papanicolaou, x40.

Right panel. Broken glands represented by strips of tall malignant columnar epithelium with basal palisading of oval, hyperchromatic nuclei containing distinct nucleolus. Papanicolaou, x400.

Figure 7  Ductal carcinoma from breast; FNAB and core biopsy of liver metastases.

(A) Cohesive clusters of monomorphous, plasmacytoid tumor cells with eccentric round nuclei. May-Grünwald-Giemsa, x200.

(B) Plasmacytoid cells show tendency to dissociation with nuclear streaking artifact. Papanicolaou, x200.
(C) Corresponding histology shows nests of a monomorphic carcinoma with suggestion of glandular differentiation. Note mitotic activity. Hematoxylin & eosin, x200

Figure 8 Acinar adenocarcinoma from prostate; FNAB of liver metastases.
Rosette-like acinar groupings of polygonal well-differentiated tumor cells with vacuolated cytoplasm and regular round nuclei. Complex capillary network holding the tumor cells together like a bunch of grapes. Papanicolaou, x200.
**Figure 9** Neuroendocrine carcinoma from pancreas; core biopsy of liver metastases with imprint cytology.

(A) Loosely cohesive aggregate of regular small round tumor cells with suggestion of rosetting and nuclear molding. May-Grünwald-Giemsa, x200.

(B) A cluster of tumor cells showing rosette formation with nuclear palisading. May-Grünwald-Giemsa, x400.

(C) Corresponding histology shows nested tumor cells with suggestion of pseudoacinar formation. Hematoxylin & eosin, x200

Inset: Chromogranin highlights the neurosecretory granules in the cytoplasm. Immunostain. x400.

**Figure 10** Combined hepatocellular-cholangiocarcinoma; liver resection.

An intimate relationship between well-differentiated adenocarcinoma composed of tubules lined by columnar epithelium and sheets of malignant hepatocytes characterized by well-defined polygonal cells displaying clear cytoplasm and central round nucleus with irregular nuclear contours. Representative sampling is an issue in small tissue samples which may not include both elements which are required for this diagnosis. Hematoxylin & eosin, x100
Figure 11 Polycystic liver disease with associated invasive adenocarcinoma; FNAB of liver.

Folded sheets of crowded lining epithelial cells with loss of honeycomb appearance. The cells exhibit increasing pleomorphism with variably-shaped nuclei, nuclear membrane irregularities, coarse chromatin, distinct nucleolus, and high nuclear-to-cytoplasmic ratio. Some dissociated tumor cells contain intracytoplasmic vacuoles. The background shows much necrotic debris. Papanicolaou, x100.

Figure 12 Moderately differentiated adenocarcinoma, primary versus metastasis; core biopsy of liver.

CK7 highlights the adenocarcinoma. Residual ductules and bile ducts are intensely stained. No biliary identifiers are discernible. Immunostain, x100.
Figure 13 Poorly differentiated adenocarcinoma, primary versus metastasis; FNAB and core biopsy of liver.

(A) Cluster of highly pleomorphic malignant cells with high nuclear-to-cytoplasmic ratio, enlarged nuclei and occasional binucleation. May-Grünwald-Giemsa, x400.

(B) Highly pleomorphic tumor cells with tendency to dissociation. The cytoplasm is fragile and vacuolated. Note “poly bag” (left) and necrosis. Papanicolaou, x200.

(C) Corresponding histology shows nests and trabeculae of tumor cells with eosinophilic cytoplasm. Hematoxylin & eosin, x100

(D) The tumor cells are immunoreactive for both CK7 (left panel) and CK20 (right panel). Hilar cholangiocarcinoma is a possibility. Immunostains, x200.
REFERENCES


