Introduction

The liver is one of the most common sites for metastatic disease, accounting for 25% of all metastases to solid organs. In the United States and Europe, secondary liver neoplasms are far more common than primary hepatic neoplasms. In the adult oncology patient, most are metastatic carcinomas, of which adenocarcinomas are the predominant subtype, followed by squamous cell carcinomas and neuroendocrine carcinomas.
Other tumor types that metastasize to the liver include melanomas, lymphomas, and rarely sarcomas.

Radiologically, metastatic disease presents as multiple liver lesions, but a solitary liver lesion in the adult oncology patient in the United States is also most likely to be a metastasis. A tissue diagnosis needs to be established before initiating diagnosis, and this is usually accomplished by image-guided sampling using fine-needle aspiration (FNA) or needle core biopsy (NCB) techniques of the liver lesion. Adequate sampling of the lesion is key to obtaining a diagnosis. Key issues for the pathologist evaluating the biopsy sample are determining the tumor type, distinguishing metastatic carcinoma from primary hepatocellular carcinoma or primary cholangiocarcinoma, and determining the site of primary origin. The pathologist uses morphology to establish a differential diagnosis and then uses semi-quantitative immunohistochemical studies to refine the diagnosis, but these techniques have limitations.

Microarray analysis and proteomic analysis have been used to establish tumor classifiers. Early studies have demonstrated the feasibility of using these on FNA and NCB samples. These technologies will improve our ability to subclassify tumors of unknown primary.

The topic of liver metastases is broad. This discussion focuses on the most significant tumor types in adult oncology patients, with an emphasis on differential diagnosis of carcinoma of unknown primary.

**Sampling Technique and Preparation**

In order to establish a treatment regimen, tissue diagnosis of liver masses is required. FNA and NCB using either transabdominal ultrasound or computed tomography scanning guidance are the most frequently used modalities and provide diagnostic specimens in over 90% of cases. Endoscopic ultrasound-guided FNA (EUS-FNA) is being used more frequently to aspirate lesions in the left lobe of the liver. The decision to use FNA or NCB depends on the size and location of the lesion, the suspected diagnosis, and the risk of complications. The experience of a radiologist performing the biopsy is also an important factor. The availability of cytologists to evaluate the FNA for adequacy may also play a role in selection of sampling technique.

A misconception among some clinicians is that NCB is better than FNA because it procures more tissue. However, studies in the literature and personal experience indicate that both are complementary, and an adequate FNA with a well-prepared cellblock can provide sufficient tissue for immunohistochemical studies.

In one recent study of 141 patients with abdominal lesions sampled with both FNA and NCB, FNA proved more sensitive than NCB at diagnosing malignancy (86.1% vs 80.6%, respectively). In other studies evaluating FNA and NCB of abdominal organs, similar results have been shown by other authors. All of these studies showed the sensitivity of FNA to be 2% to 24% greater than that of NCB. The combination of FNA and NCB increased the overall sensitivity. A few studies have provided results contradicting these findings. However, none of these studies used on-site immediate assessment of FNA samples by a cytologist, which has been shown to maximize diagnostic yield and accuracy.

A well-prepared cellblock derived from an FNA sample produces a microhistology specimen that can provide sufficient material to evaluate for architectural features and to perform immunohistochemical studies. This provides an alternative to NCB when an NCB cannot conveniently be performed. If only an NCB will be obtained, then cytologic evaluation of touch preparations of the cores can provide similar rapid assessments of specimen adequacy. Touch imprints of core biopsies may be used to minimize the number of biopsy procedures needed by ensuring that the NCB contains diagnostic material.

In summary, selection of guidance and biopsy technique depends on the location and site of the lesion, the potential for complications using either technique, and the experience and expertise of the radiologist. FNA and NCB are complementary. On-site cytologic assessment of FNA or NCB using touch imprints improves the adequacy of both.

**Establishing the Tumor Type**

Correct clinical history is crucial to the pathologic interpretation of a biopsy from a liver mass suspected of being a metastasis. The most accurate interpretation is rendered when the history of previous cancer and other pertinent findings, such as radiological findings and serum tumor marker levels, are provided for correlation with the pathologic findings.

The first step when evaluating a liver biopsy is to establish the general tumor type, ie, whether the neoplasm is a carcinoma, sarcoma, lymphoma, or melanoma. Morphology alone is often diagnostic, but frequently ancillary studies are needed for definitive diagnosis when the tumor is poorly differentiated. A panel incorporating at least cytokeratins, S100, and leukocyte-common antigen (LCA) will assist in subcategorizing the neoplasms. Carcinomas express cytokeratins, most lymphomas (except anaplastic large-cell lymphoma) express LCA, and S100 is the most sensitive marker for the diagnosis of melanoma. Additional antibodies can be added once the differential diagnosis has been narrowed down.

**Carcinomas**

Carcinomas are the most frequent source of metastases to the liver. Lung, colon, pancreas, breast, and stomach...
are the most frequent sources, accounting for 24.8%, 15.7%, 10.9%, 10.1% and 6.1%, respectively, of all patients with metastatic disease in one autopsy series.²¹

Ovarian, endometrial, prostate, and urothelial carcinomas are less frequent sources of metastases, each accounting for 4% or less.²¹

The appearance of carcinomas depends on their differentiation and includes adenocarcinomas, squamous cell carcinoma, urothelial carcinoma, neuroendocrine carcinomas, mixed types of carcinomas such as adenosquamous carcinoma, or specific types such as adrenal cortical carcinoma, renal cell carcinoma, and hepatocellular carcinoma.

**Squamous Cell Carcinoma**

Squamous cell carcinoma is an uncommon metastasis to the liver. Possible primary sites include lungs, esophagus, head and neck, genital primaries, or anorectal primaries. FNA smears show polygonal cells occurring singly and in groups with hyperchromatic, irregular nuclei. The cytoplasm is dense and nonvacuolated, in contrast to that of adenocarcinoma (Fig 1). Keratinized cells will have orangeophilic cytoplasm on Papanicolaou-stained smears. Histopathology specimens will show cells with dense, polygonal, eosinophilic cytoplasm with intercellular desmoplastic junctions (Fig 2). The presence of keratinization confirms the diagnosis, but it is not always evident. The morphology of squamous cell carcinoma is not specific to site of origin. History is key in identifying the primary site since immunohistochemical studies are not helpful.

**Urothelial Carcinoma**

The cells on cytology smears are arranged in discrete and small syncytial cell clusters. The nuclei are central to eccentric, and the cytoplasm is variable. A key feature is the presence of cercariform cells (Fig 3). These are cells with nucleated globular bodies and unipolar nontapering cytoplasmic process.²² The pattern on histomorphology is varied. The cells are typically arranged in sheets and have dense, amphophilic cytoplasm. The nuclei are typically elongated and may show grooves (Fig 4).
Neuroendocrine Carcinomas

Neuroendocrine carcinomas vary in the degree of differentiation. Low-grade tumors such as carcinoids have a monomorphic appearance with minimal mitotic activity and no necrosis. Smears are uniformly cellular and composed of a monomorphic population of tumor cells. A tumor with a plasmacytoid appearance on cytology samples is virtually pathognomonic (Fig 5). Histopathology samples will show similar features. The chromatin shows a characteristic salt and pepper appearance on both smears and core biopsy samples (Fig 6). Carcinoids may arise anywhere in the gastrointestinal tract. Pancreatic endocrine tumors have identical morphologic features. High-grade tumors, such as metastatic small-cell carcinoma from the lung, will show nuclear molding, necrosis, and abundant mitotic activity. The chromatin is diffusely and finely stippled (Figs 7 and 8).

Immunohistochemical studies are useful for the identification of a neoplasm as showing neuroendocrine differentiation. The standard panel is synaptophysin, chromogranin, and neural cell adhesion molecules (NCAM [CD56]). Immunohistochemical studies are less helpful for the identification of neuroendocrine carcinoma of unknown primary.23

Adenocarcinomas

Adenocarcinomas are the most significant since they are the most frequent type of carcinoma to metastasize to the liver. Lung, colon, pancreas, breast, and stomach are the most frequent, representing 24.8%, 15.7%, 10.9%, 10.9%, and 6.1% of cases, respectively, in one autopsy series.21 Ovary, endometrial, prostate, cholangiocarcinoma, and thyroid are less frequent, each accounting for less than 4%.21 Adenocarcinomas are also the most frequent type of carcinoma presenting as unknown primary in the liver in the adult oncology patient.24,25

Adenocarcinomas are neoplasms derived from glandular tissues. The most frequent appearance of adenocarcinomas is columnar cells forming acinar structures, which recapitulate the gland formation within...
the normal organ (Fig 9). The typical adenocarcinoma shows focal mucin production, within either the cytoplasm or lumen, which can be demonstrated with a histochemical stain for mucin, such as mucicarmine.

Morphologic subtypes include the mucinous carcinoma or colloid-type carcinoma and signet ring cell carcinoma. While the morphologic pattern of most adenocarcinomas is not specific for site of origin, some primary sites have characteristic features that lead to their recognition on FNA or NCB specimens. These include colorectal carcinoma, breast carcinoma, and pancreatobiliary carcinoma.

A key feature of colorectal carcinoma is a dirty, necrotic background on cytology smears (Fig 10). The cells are columnar in appearance. Histopathology will show an adenocarcinoma with abundant central necrosis in the glands (Fig 11). Low-grade ductal adenocarcinomas appear as a monomorphic population on cytology smears. The groups are flat and angulated. The monomorphic appearance is evident on histopathology specimens (Fig 12). Lobular carcinoma forms a dyshesive cell population composed of small cells with eccentric nuclei. Histology specimens will show cells infiltrating in single file pattern. A characteristic feature of both types of mammary carcinomas, associated most often with lobular carcinoma, is cells with a targetoid cytoplasmic lumen (Fig 13).

Pancreatobiliary carcinomas do not exhibit a specific pattern on cytology smears, although an adenocarcinoma with abundant cytoplasmic mucin or clear nuclei may be suggestive. They are typically associated with abundant sclerotic stroma (Fig 14), and therefore this possibility may be suggested on histopathology specimens. However, primary intrahepatic cholangiocarcinoma is also associated with sclerotic stroma, so the distinction of primary cholangiocarcinoma from metastatic pancreatobiliary carcinoma cannot be made without the history.

The morphologic patterns of other types of adenocarcinomas such as those originating in the lungs, endometrium, esophagus, or intestinal type of gastric carcinoma do not have any specific features.

![Fig 9. — Adenocarcinoma, NOS, core biopsy. The cells are arranged in an acinar pattern, characteristic of adenocarcinoma from any site. Pale, bluish mucin is evident the cytoplasm of some cells and lumen (hematoxylin-eosin, × 40).](image)

![Fig 10. — Colonic adenocarcinoma, FNA. The smear shows columnar cells arranged in a palisaded pattern. The background shows abundant necrosis (Papanicolaou, × 40).](image)

![Fig 11. — Colonic adenocarcinoma, core biopsy. The glands show abundant central necrosis, a characteristic feature of colonic adenocarcinoma (hematoxylin-eosin, × 40).](image)

![Fig 12. — Mammary carcinoma, FNA. The cells are dispersed and show eccentric nuclei. A number of cells have intracytoplasmic mucin vacuoles (Diff-Quik, × 60).](image)
**Mucinous Carcinoma**

Mucinous carcinoma is a morphologic subtype of adenocarcinoma, defined as a carcinoma that contains more than 50% extracellular mucin. This subtype is not specific to any organ. Mucinous carcinomas are most frequently identified in the colon, but they also occur in the breast, ovaries, and pancreas and may arise anywhere in the gastrointestinal tract. Mucinous bronchoalveolar carcinoma has similar features. A mucinous carcinoma in the liver is most likely to be of colorectal origin, but other primary sites need to be considered. Aspirates show malignant glandular cells floating in pools of mucin (Fig 15). The two histologic patterns are tumor cells floating in pools of mucin or pools of mucin partially lined by tumor cells (Fig 16). The tumor cells are mucin-producing columnar cells or cells that contain a single large vacuole.

**Signet Ring Cell Carcinoma**

Signet ring cell carcinoma shows single cells with a cytoplasmic mucin vacuole that displaces the nucleus. The nucleus is sharply angulated at the tips (Fig 17). Gastric carcinoma is most commonly associated with this morphology, but as for mucinous carcinomas, it may arise in any organ in the gastrointestinal tract. The differential diagnosis includes metastatic lobular breast carcinoma. An immunohistochemical panel that

![Fig 13](image-url) — Mammary carcinoma, core biopsy. The cells are arranged in rounded nests (hematoxylin-eosin, × 40).

![Fig 14](image-url) — Metastatic pancreatic carcinoma. The carcinoma is surrounded by a desmoplastic stroma (hematoxylin-eosin, × 20).

![Fig 15](image-url) — Mucinous carcinoma, FNA. The cluster of malignant cells floats in a background of viscous mucin (Papanicolaou, × 40).

![Fig 16](image-url) — Mucinous carcinoma, biopsy. The carcinoma is composed predominantly of extra cellular mucin. The malignant glands cling to the stroma and surround pools of mucin (hematoxylin-eosin, × 20).

![Fig 17](image-url) — Signet ring cell carcinoma, core biopsy. Signet ring cells with eccentric, elongated nuclei displaced by intracytoplasmic mucin vacuoles (hematoxylin-eosin, × 40).
includes CK7, CK20, estrogen receptors, and GCDFP15 (BRST2) can help with this differential diagnosis. Lobular carcinoma will express CK7, ER, and GCDFP15 and usually does not express CK20. Progesterone receptor is not as specific as estrogen receptor since its expression was identified in other carcinomas, including gastric/esophageal.

**Adenosquamous Carcinoma**

Adenosquamous carcinoma is composed of a mixture of squamous carcinoma and adenocarcinoma, as the name implies. One of the components must comprise at least 30% of the tumor. This type of carcinoma may arise anywhere in the gastrointestinal tract, including the pancreas and biliary system, and also the lungs. Since primary cholangiocarcinoma may show a similar morphology, it will not be possible to determine whether it is primary or metastatic without clinical history.

**Renal Cell Carcinoma**

Renal cell carcinoma infrequently metastasizes to the liver, but it accounts for 3% of all metastases in one autopsy series. The classic appearance is that of a carcinoma composed of clear cells arranged in nests with intervening stroma and blood vessels (Fig 18). Variants include papillary renal cell carcinoma and chromophobe renal cell carcinoma. The cytoplasm of FNA will show polygonal cells arranged singly and in clusters. The nuclei are round with prominent nucleoli, and the cytoplasm is clear or granular. Large groups or sheets of cells are arranged along transgressing endothelium (Fig 19), a pattern that mimics hepatocellular carcinoma on aspirates. Papillary renal cell carcinoma will not demonstrate prominent nucleoli or clear cytoplasm. The cytoplasm in chromophobe renal cell carcinoma is balloon-like and excessive. The pattern of clear cell renal cell carcinoma may mimic that of hepatocellular carcinoma, particularly the clear cell variant of hepatocellular carcinoma.

**Adrenal Cortical Carcinoma**

Adrenal cortical carcinoma is a rare neoplasm. The liver is one of its most common sites of metastasis. It merits mention because its morphologic pattern overlaps...
with that of hepatocellular carcinoma on FNA and NCB. The cells of adrenal cortical carcinoma are polygonal in shape, like those of hepatocellular carcinoma. Furthermore, on core biopsy samples, it may show endothelial wrapping, similar to that produced by hepatocellular carcinoma (Figs 20 and 21). Smears show polygonal cells arranged singly and in clusters without transgressing endothelium. The nuclei are hyperchromatic and variable in size. Prominent nucleoli are not a feature as they are for hepatocellular carcinoma.

**Melanomas**

Melanomas are known as the great mimickers in pathology. Generally, melanoma must be included in the differential diagnosis of almost any neoplasm since its appearance can be so varied and also since patients may present with liver metastases many years after the diagnosis of the primary tumor. However, it is a relatively infrequent source of metastases in the liver, accounting for approximately 2.2%. A single cell population with cytoplasmic melanin pigment is diagnostic on FNA smears (Fig 22), but nonpigmented or amelanotic melanomas are difficult to diagnose. Biopsies typically show a population of pleomorphic cells with intranuclear inclusions and prominent nucleoli (Fig 23). Immunohistochemistry is specific and sensitive for the diagnosis of melanoma. Melanomas are S100-positive, HMB45 and MelanA-positive. A new antigen cocktail consisting of HMB45, tyrosinase, and MART-1 is sensitive for the diagnosis of melanoma. However, S100 remains the most sensitive for detection of melanoma, although it lacks specificity.34

**Lymphomas**

Lymphomas are dyshesive neoplasms on FNA smears. The background of the smears shows lymphoglandular bodies (Fig 24) indicating that the neoplasm is of lymphoid origin, but they are not specific for benignancy or malignancy. The appearance depends on the specific type. Large-cell lymphomas, the most frequent type of lymphoma to secondarily involve the liver, are usually easy to recognize as malignant because they are composed of large lymphocytes (greater than 3 times the size of a normal lymphocyte) with nuclear membrane irregularities (Fig 25). Follicular lymphomas, mucosa-

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**Fig 22.** — Melanoma, FNA. The smear shows a dispersed cell population. The nuclei vary in size and shape. Some of the cells show black melanin pigment in the cytoplasm (Diff-Quik, × 40).

**Fig 23.** — Melanoma, core biopsy. The malignant cells have atypical nuclei with prominent nucleoli, a feature considered typical of melanoma. Melanin pigment is visible (hematoxylin-eosin, × 40).

**Fig 24.** — Large cell lymphoma, FNA. The malignant cells are dispersed. The nuclei are three times the size of normal lymphocytes. The background shows lymphoglandular bodies, which are characteristic for lymphoid processes (Diff-Quik, × 63).

**Fig 25.** — Large cell lymphoma, biopsy. The specimen shows a population of cells with scant cytoplasm, irregular nuclear membranes and prominent nucleoli (hematoxylin-eosin, × 40).
associated lymphoma tissue (MALT) lymphomas, and small lymphocytic lymphomas are difficult to recognize on morphology alone but can be suspected if there is a previous history of lymphoma. Flow cytometry and gene rearrangement studies can be performed on samples obtained from FNA. Typically, if lymphoma is suspected, an aspirate directed for flow cytometry is requested. NCB can be used for histomorphological grading of follicular lymphomas and immunohistochemical studies. The accuracy of subclassifying lymphomas using cytomorphological and flow cytometric immunophenotyping has been demonstrated.35-37

**Sarcomas**

Sarcomas are usually characterized by a spindle cell appearance. Gastrointestinal stromal tumors and leiomyosarcomas are the most frequent sarcomas to metastasize to the liver.

Aspirates of gastrointestinal stromal tumors demonstrate relatively monomorphic and uniform spindle cells in loose aggregates and singly. The cells may be associated with a myxoid stroma (Fig 26). Occasionally they show epithelioid features, in which case the differential diagnosis includes melanoma, carcinomas, and neuroendocrine tumors. Histopathology samples demonstrate a spindle cell neoplasm with a storiform pattern, prominent vascularity, and occasionally skenoid fibers (Fig 27). The tumors may demonstrate variable amounts of myxoid stroma. Paranuclear vacuoles are a frequent feature (Fig 28). Immunohistochemical analysis shows tumor cell expression for C-kit, CD34, and vimentin. The tumors are typically negative for actin or desmin.38 C-kit expression is diagnostic for these tumors.

Leiomyosarcoma is the most common sarcoma to metastasize to the liver. It shows greater pleomorphism and less vascularity compared to gastrointestinal stromal tumor (GIST) (Fig 29). The characteristic immunophenotype is desmin and smooth muscle actin expression and no C-kit expression.38

**Diagnostic Dilemmas**

Problems facing the pathologist when evaluating a biopsy include distinguishing hepatocellular carcinoma from other carcinomas with similar features (renal cell
and adrenal cortical carcinoma) and distinguishing hepatocellular carcinoma from adenocarcinoma. A special problem in the oncology patient is the workup of carcinoma of unknown primary.

**Primary Hepatocellular Carcinoma vs Metastases**

The morphologic features of renal cell carcinoma and adrenal cortical carcinoma overlap with those of hepatocellular carcinoma, on both cytology smears and histopathology samples. Immunohistochemical analysis differentiates among these three carcinomas. Adrenal cortical carcinomas are weakly positive for cytokeratin and express vimentin and synaptophysin. MART-1, also known as melanA, and usually expressed by melanoma, is useful for diagnosing adrenal cortical carcinoma. Adrenal cortical carcinoma also expresses inhibin, which is slightly more sensitive but less specific. Calretinin has also been shown to be expressed by adrenal cortical cells and neoplasms. Clear cell carcinoma of the kidney, the most typical type of renal neoplasm, expresses cytokeratin, vimentin, epithelial membrane antigen (EMA), and CD10. Exceptions are the papillary variants and chromophobe cell variants, which do not express vimentin. Alpha-methyl CoA racemase (AMACR) is usually expressed by papillary renal cell carcinomas, and chromophobe renal cell carcinomas demonstrate colloidal iron not demonstrated by hepatocellular carcinoma or adrenal cortical carcinoma. Hepatocellular carcinoma can usually be identified by expression of low-molecular-weight cytokeratin, HepPar, and a canalicular pattern rather than a cytoplasmic pattern of carcinoembryonic antigen (CEA). The canalicular pattern occurs because the CEA will stain the bile duct canaliculi in hepatocellular carcinoma but not the cytoplasm. CD10 will stain hepatocellular carcinoma in a canalicular pattern but not in the cytoplasm, as it does for renal cell carcinoma.

Other unusual metastases that may mimic hepatocellular carcinoma include hepatoid yolk sac tumor, and oxyphilic follicular carcinoma of the thyroid. Hepatoid yolk sac tumors are rare, and follicular carcinoma of the thyroid rarely gives rise to liver metastases. The immunohistochemical approach to this differential diagnosis is summarized in Table 1.

**Adenocarcinoma vs Hepatocellular Carcinoma**

Typically, the distinction of adenocarcinoma from hepatocellular carcinoma is clear-cut on morphologic grounds. Adenocarcinoma is characterized by the formation of gland-like or tubular structures. The lumens or the individual cells contain mucin. Hepatocellular carcinoma occasionally forms acinar structures resembling adenocarcinoma (Fig 30) or is poorly differentiated, in which case it cannot be distinguished from adenocarcinoma. In these situations, adjunctive studies are needed. The presence of bile pigment is pathognomonic for hepatocellular differentiation; therefore, if

![Figure 30](image_url)
the pathologist recognizes bile, the diagnosis of hepatocellular carcinoma can be made with certainty. A Hall's stain for bile can help to recognize the bile pigment. Adenocarcinoma typically secretes mucin, so the identification of mucin secretion by the cells using a histochemical stain for mucin, such as mucicarmine, can establish the carcinoma as an adenocarcinoma.

Immunohistochemistry is helpful when morphology and identification of secretory substances fail. Table 2 lists an immunohistochemical panel to facilitate this distinction. Adenocarcinomas express both high- and low-molecular-weight cytokeratin, whereas the cytokeratin expression of hepatocellular carcinoma is usually limited to low-molecular-weight cytokeratin. More specifically, hepatocytes and hepatocellular carcinoma do not express cytokeratins 1, 5, 10, 11, and 19. Immunohistochemical evaluation for CK19 is particularly useful since adenoscarcinomas but not hepatocellular carcinomas express this antigen. As described in the previous section, hepatocellular carcinoma has a specific expression pattern for CEA and CD10 in which they are expressed only in the bile canaliculi. Adenocarcinomas show a cytoplasmic expression pattern for CEA. MOC31 is reported to be sensitive for the diagnosis of adenocarcinoma. The HepPar antigen is also helpful, but it is expressed occasionally by other tumor types such as mucicarmine, can establish the carcinoma as an adenocarcinoma.

Table 2. — Differential Diagnosis of Hepatocellular Carcinoma From Adenocarcinoma

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Hepatocellular Carcinoma</th>
<th>Adenocarcinoma</th>
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<tr>
<td>LMW CK</td>
<td>+</td>
<td>+</td>
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<tr>
<td>HMW CK</td>
<td>–/rarely +</td>
<td>+</td>
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<tr>
<td>CEA</td>
<td>+</td>
<td>–</td>
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<tr>
<td>HepPar</td>
<td>+</td>
<td>–</td>
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<tr>
<td>B72.3</td>
<td>–</td>
<td>–</td>
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<tr>
<td>AFP</td>
<td>+</td>
<td>–</td>
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<tr>
<td>MOC31</td>
<td>–</td>
<td>–</td>
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<tr>
<td>CK19</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Mucicarmine</td>
<td>+</td>
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<td>Bile</td>
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<tr>
<td>LMW CK</td>
<td>low-molecular-weight cytokeratin</td>
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Primary Site of Origin of Carcinoma

This problem is probably of the greatest significance to the oncologist treating the adult cancer patient and is frequently encountered at our institute. While most patients presenting with metastatic adenocarcinoma have a history of a primary elsewhere, some patients do not have a known primary. In these cases, the onus falls on the pathologist to navigate the treating physicians to the most likely primary site.

As previously stated, adenocarcinomas are the most frequent type of carcinoma presenting as unknown primary, accounting for approximately 80%. Squamous cell carcinomas account for another 15%, and metastases of other tumor types account for the remaining 5%. The immunohistochemical workup essentially focuses on adenoscarcinomas since the immunohistochemical panels are most applicable to this subtype of carcinoma.

Morphologic clues on the FNA or NCB may help to distinguish some adenoscarcinomas from others, as already presented. In a patient with a solitary liver mass, the key will be distinguishing primary cholangiocarcinoma from metastatic adenocarcinoma. Morphology may provide some clues since the association with a densely sclerotic stroma characterizes cholangiocarcinoma, and an origin or close interconnection with adjacent canals of Herring may be seen. Except for some of the morphologic types already mentioned, morphology is otherwise not useful for identification of carcinoma of unknown primary.

Immunohistochemistry

The limitations of morphology have fueled the search for markers of differentiation. Since the first major publication reporting cytokeratin phenotype of CK7 and CK20 as discriminatory among different tumor types and sites, it has become the cornerstone of the panel to evaluate tumors of unknown origin. There are four possible expression patterns: CK7+/CK20+, CK7+/CK20−, CK7−/CK20+, and CK7−/CK20−. One difficulty with interpreting the CK7/CK20 phenotype is that the criteria used to define a tumor as positive for antigen expression have varied significantly among authors. Some authors have required only 1% of cells while others have required at least 50%. Adding to the difficulties in interpreting the findings is that the antibodies used are different and that there are differences in antigen retrieval techniques and performance of the immunohistochemical studies. The CK7/CK20 phenotype is also influenced by the degree of differentiation and the morphologic subtype. All of these caveats mean that the pathologic interpretation of CK7/CK20 immunohistochemical studies remains subjective, but it remains most effective when based on an algorithmic and probabilistic approach. However, despite these limitations, the CK7/CK20 expression pattern is effective at narrowing down possibilities.

When applying an algorithmic approach to identifying the origin of an adenocarcinoma, the first step involves distinguishing the primary from a metastasis. In the case of cholangiocarcinoma, the diagnosis remains one of exclusion in most cases because its phenotype overlaps with that of many other carcinomas.
Cholangiocarcinomas are usually CK7+ and variably express CK20.59 Their morphologic expression pattern overlaps with the pattern of many other primary sites. The most relevant phenotype to the discussion of metastatic adenocarcinoma to the liver is the CK7−/CK20 + phenotype because it is highly characteristic of colorectal primary.61 The predictive probability of this immunophenotype is 78%.62 Carcinomas coexpressing CK7/CK20 include urothelial carcinoma, metastatic pancreatobiliary carcinoma, and mucinous ovarian carcinoma.58,63 Recent publications have shown that mucinous colon carcinoma and mucinous bronchoalveolar carcinoma also express the CK7+/CK20+ phenotype.64-67 Morphology can sometimes exclude metastatic urothelial carcinoma since this is not a gland-forming neoplasm; however, its features may overlap with those of poorly differentiated adenocarcinoma. The CK7−/CK20− phenotype is usually typical of prostate, with a probability of 76%.62 Other tumors rarely show this phenotype. The CK7+/CK20− subtype is the least specific.63 Lung and breast are two tumors that exclusively have this phenotype, with a probability of 84% and 88%, respectively. However, other tumors can express this phenotype, particularly if CK20 expression is weak or focal. Of note, gastric and esophageal adenocarcinoma have the most variable CK7/CK20 expression pattern.58,61 Therefore, these need to be included in the differential diagnosis of any phenotype. Table 3 summarizes the most frequent CK7/CK20 expression patterns for carcinomas of different primary sites.

Other studies can help to further refine the differential diagnosis. Cytokeratin 17 is associated with ampullary and pancreatobiliary carcinomas more often than gastric or esophageal carcinomas.58,69 Additional markers help to further refine the identification such as TTF1 (nonmucinous pulmonary adenocarcinomas),64-70 estrogen receptor protein staining (breast, ovarian, endometrial),29,75 GCDFP15 (breast),27,76-78 WT1 (ovarian serous tumors),79 PSA and PAP (prostate),80 thrombomodulin,81 and uroplakin (urothelial carcinoma).80,82 CA125, when used as part of a panel, is useful for identifying ovarian carcinomas.83,84 CDX2 is used as a marker of intestinal differentiation and can help to differentiate colorectal, intestinal, or gastric neoplasms from pancreatobiliary, biliary, ovarian, or pulmonary adenocarcinomas.65,85-87

Table 3. — Cytokeratin Coexpression Patterns

<table>
<thead>
<tr>
<th>CK7+/CK20+</th>
<th>CK7+/CK20−</th>
<th>CK7−/CK20+</th>
<th>CK7−/CK20−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urothelial carcinoma, breast, colorectal, prostate, renal cell carcinoma, hepatocellular carcinoma, adrenal cortical carcinoma</td>
<td>Lung, esophagus/stomach, pancreas, biliary, Cholangiocarcinoma, Ovary (nonmucinous), Endometrium</td>
<td>Pancreas, Cholangiocarcinoma, Ovary (nonmucinous)</td>
<td>Prostate, Renal cell carcinoma, Hepatocellular carcinoma, Adrenal cortical carcinoma</td>
</tr>
</tbody>
</table>

An older antibody panels consisting of MOC31, keratins, vimentin, B72.3, CA125, Ca19-9, placental alkaline phosphatase, S100 protein, estrogen receptor protein, PSA, thyroglobulin, GCDFP15, and CEA obtained a sensitivity of 67% for the diagnosis of carcinoma of unknown primary. Another study evaluating GCDFP15, breast cancer antigen 225 (BCA225), B72.3, CA15-3, CEA, CA19-9, CA125, and estrogen receptor showed a sensitivity of 67% for the determination of site of origin.89 While individual studies have evaluated the sensitivity and specificity of sets of markers for specific differential diagnoses, the sensitivity of a panel including CK7 and CK20 with some of the above listed antibodies has not been determined across a broad number of cancers in the liver.

Future Directions

Cancer therapy is primarily directed by tumor origin, making correct pathologic diagnosis imperative for proper patient management. As discussed, the number of specific markers available and subjectivity of interpretation are factors that limit standard pathologic practice using morphology and immunohistochemistry. Tumor classification, using high throughput technologies such as microarray and proteomic screening, promise to improve cancer diagnosis and management.

A number of publications have demonstrated the feasibility of using gene expression profiles derived from microarray analysis as an approach to diagnosis.90-92 Proteomic analysis has the potential to yield similar data sets.93

In order for these technologies to be clinically relevant, they must be applicable with FNA and NCB. Recent studies have shown the feasibility of using FNA or NCB specimens for microarray analysis.94-99 In our own experience, FNA of resection specimens obtained over 1 μg of total RNA for microarray analysis.100 The samples were successfully classified using a tumor classifier derived from resection specimens. Studies evaluating the use of cytology or NCB material for proteomic analysis are limited.101
The problem for any of these technologies is the lack of standardization in specimen collection and preparation. However, these technologies promise to supplement and improve our current standard of practice as adjunctive techniques.

References


