

# Expression of Cytokeratins 7 and 20 in Carcinomas of the Extrahepatic Biliary Tract, Pancreas, and Gallbladder

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● **Background.**—Expression of cytokeratins 7 (CK7) and 20 (CK20) may help distinguish the site of origin for metastatic carcinomas. Little is known regarding their expression in biliary tract and pancreatic carcinomas. Our aim was to study the expression of CK7 and CK20 in these tumors.

**Design.**—Fifty-three carcinomas of the extrahepatic bile ducts (n = 8), ampulla of Vater (n = 7), gallbladder (n = 11), and pancreas (n = 27), were retrieved from the surgical pathology files of the University of Massachusetts Medical Center. Formalin-fixed, paraffin-embedded sections were immunostained with mouse monoclonal antibodies to CK7 and CK20 using an avidin-biotin immunoperoxidase technique with microwave antigen retrieval.

Cytokeratins are well-described intermediate filament proteins of both normal epithelia and epithelial tumors. Antibodies to several different subtypes of cytokeratin have been available for some time, including cytokeratin 7 (CK7)<sup>1</sup> and cytokeratin 20 (CK20).<sup>2</sup> In normal tissues, CK7 is typically found in simple epithelia from the gastrointestinal tract<sup>3,4</sup> (including the gallbladder,<sup>1</sup> hepatic ducts,<sup>1,5–10</sup> and pancreatic ducts<sup>11</sup>), female genital tract (the endometrium and fallopian tube), breast,<sup>1</sup> urinary tract (bladder),<sup>1</sup> and respiratory tract (lung).<sup>1,12</sup> In contrast, CK20 is found in more complex epithelia from the gastrointestinal tract (such as the gastric and intestinal mucosa), genitourinary tract (urothelium), squamous epithelia from any site, and Merkel cells.<sup>2,3</sup> Because these cytokeratins usually retain their tissue specificity in their neoplastic counterparts, coordinate expression of these 2 cytokeratins has recently been proposed to help identify the site of origin of various metastatic carcinomas.<sup>13–15</sup>

Identifying the tissue of origin for tumors arising in and around the biliary tract is particularly problematic for the pathologist, due in part to the anatomy of the region. The close proximity of the gallbladder, bile ducts, and pancreas is further compounded by the histologic similarity and the locally aggressive behavior of their carcinomas. Given the apparent success in using cytokeratin expression to identify the site of origin for many metastatic carcinomas,

The percentage of cells positive for each antibody was assessed on a scale of 0 to 3 (0, <10%; 1+, 10% to 50%; 2+, 51% to 90%; 3+, >90%).

**Results.**—The majority of carcinomas in all groups were positive for CK7 (CK7+) and negative for CK20 (CK20–). Of the CK7+ tumors, the majority of tumors in each group were 3+ positive.

**Conclusions.**—(1) Carcinomas of the extrahepatic biliary tract and pancreas are strongly positive for CK7 and negative for CK20 and can be included in the differential diagnosis of other carcinomas with this profile in metastatic sites. (2) The CK7/CK20 immunostaining profile will not identify the site of origin for tumors with extensive growth in the porta hepatis region.

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we questioned whether this approach could be used to identify carcinomas of the biliary tract or pancreas in metastatic sites, or to pinpoint the exact site of origin for carcinomas with extensive growth in the porta hepatis.

## METHODS

### Cases

Carcinomas of the extrahepatic bile ducts (EHBD), ampulla of Vater, gallbladder, and pancreas were obtained from the surgical pathology files of the University of Massachusetts Medical Center from 1990 through 1998. Fifty-three cases were identified, including 8 carcinomas of the EHBD (1 hepatic duct, 7 common bile duct), 7 ampullary carcinomas, 11 carcinomas of the gallbladder, and 27 carcinomas of the pancreas (24 pancreatic ductal and 3 cystadenocarcinomas). All cases were resected primary tumors with the exceptions of 1 resected liver metastasis and 1 resected small bowel metastasis, both from known primary gallbladder carcinomas. Biopsy specimens were not included in the study. The routine H&E slides were reviewed and 1 representative section was chosen for immunostaining.

### Immunostaining

Paraffin-embedded, formalin-fixed tissue blocks were cut at 4  $\mu$ m, heated at 60°C for 30 minutes, then deparaffinized and hydrated through a series of xylenes and alcohols. Optimum pretreatment and dilutions were determined by testing with both known-positive and known-negative material. Mouse monoclonal antibodies against CK7 (Dako Corporation, Carpinteria, Calif) at a dilution of 1:200 and CK20 (Dako) at a dilution of 1:100 with antigen retrieval gave us the best signal-to-noise ratio in the positive control sections and no staining in the negative control sections. The slides were microwaved with a proprietary antigen retrieval solution (citrate buffer, BioTek Solution, BioTek, Santa Barbara, Calif) for 5 minutes in an 800-W microwave oven. Following replenishment of this solution, the slides were micro-

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waved again for an additional 5 minutes and then allowed to cool for 20 minutes. The slides were stained on a BioTek solutions TechMate 1000 automated immunostainer using an avidin-biotin complex (ABC) staining procedure (BioTek). Following a hydrogen peroxide block of endogenous peroxidase and a serum blocking step, the slides were incubated with the primary antibody for 45 minutes followed by brief buffer washes, then incubated in a cocktail of biotinylated antimouse IgG and IgM and rabbit IgG (BioTek) for 30 minutes. The sections were then washed, incubated in ABC for 30 minutes, washed, then reacted with diaminobenzidine and hydrogen peroxide to visualize the end product. Sections were counterstained with hematoxylin. A duplicate set of slides was also stained in the exact same manner substituting normal mouse serum for the CK7 or CK20 antibody to serve as a negative control.

### Evaluation of Immunostaining

Positive immunostaining for CK7 and CK20 was identified as unquestionable brown staining in the cytoplasm, the cell membrane, or both compartments of tumor cells. A semiquantitative method was used to assess the distribution of immunostaining. An estimate of the number of positive cells was made in each case in the following categories: 0, no or only rare single cells positive; 1+, up to 50% of tumor cells positive; 2+, between 51% and 90% of tumor cells positive; and 3+, more than 90% of tumor cells positive. For analysis of the data by tumor, a tumor was considered "positive" for CK7 or CK 20 if the immunostaining result was 1+, 2+, or 3+.

### RESULTS

The positive staining for both CK7 and CK20 localized to the cytoplasm of tumor cells in all cases. Examples of positive and negative staining in carcinomas of the gallbladder, bile ducts, and pancreas are illustrated in Figure 1. The number of tumors positive for CK7 and CK20 is shown by grade for each tumor type in Figures 2 and 3.

#### Carcinoma of the Extrahepatic Bile Ducts

The 8 EHBD carcinomas were obtained from 6 men and 2 women, ages 31 years to 77 years. They included 2 grade I (well-differentiated), 3 grade II (moderately differentiated), and 1 grade III (poorly differentiated) invasive carcinomas and 2 in situ carcinomas. One invasive carcinoma was confined to the bile duct wall, 2 invaded adjacent tissues, and 3 had liver or lymph node metastases. Seven tumors (88%) stained positive for CK7 (CK7+) and only 2 tumors (25%) stained positive for CK20 (CK20+). Both CK20+ tumors were also CK7+. There was no apparent correlation between expression of CK7 and tumor grade or stage, since all but 1 tumor were CK7-positive.

#### Carcinoma of the Ampulla of Vater

The 7 carcinomas were obtained from 3 men and 4 women, ages 57 years to 80 years. They included 2 grade I, 4 grade II, and 1 grade III tumors. Only 1 tumor was confined to the ampulla; 2 others were locally invasive, and 4 had lymph node metastases. Six tumors (86%) were CK7+, all 3+, and all 7 were negative for CK20 (CK20-). There was, thus, no correlation between expression of CK7 and tumor grade or stage.

#### Carcinoma of the Pancreas

The 27 pancreatic carcinomas were obtained from 13 men and 14 women, ages 51 years to 82 years. They included 2 grade I, 23 grade II, and 2 grade III tumors. One was carcinoma in situ (stage 0), 10 were confined to the pancreas or locally invaded the adjacent duodenal wall

(stages I or II), and 16 exhibited lymph node metastases (stages III or IV). Twenty-six tumors (96%) were CK7+ and 2 (7%) were CK20+. Of the CK20+ tumors, 1 was CK7+ and the other was negative for CK7 (CK7-). There was no apparent correlation between expression of CK7 and tumor stage or grade.

#### Carcinoma of the Gallbladder

The 11 gallbladder carcinomas were obtained from 3 men and 8 women, ages 58 years to 85 years. They included 5 grade I and 6 grade II tumors. No poorly differentiated (grade III) tumors were identified. One tumor was carcinoma in situ (stage 0), 3 were confined to the gallbladder (stage I or II), 2 locally invaded the adjacent tissues (stage III or IV), 3 metastasized to lymph nodes (stage III or IV), and 2 exhibited distant metastases (stage IV). Nine tumors (82%) were CK7+ and 3 (27%) were CK20+. Of the 3 CK20+ tumors, 2 were also CK7+, and 1 was CK7-. There was no apparent correlation between expression of CK7 and tumor grade or stage.

#### Coordinate Expression of CK7 and CK20

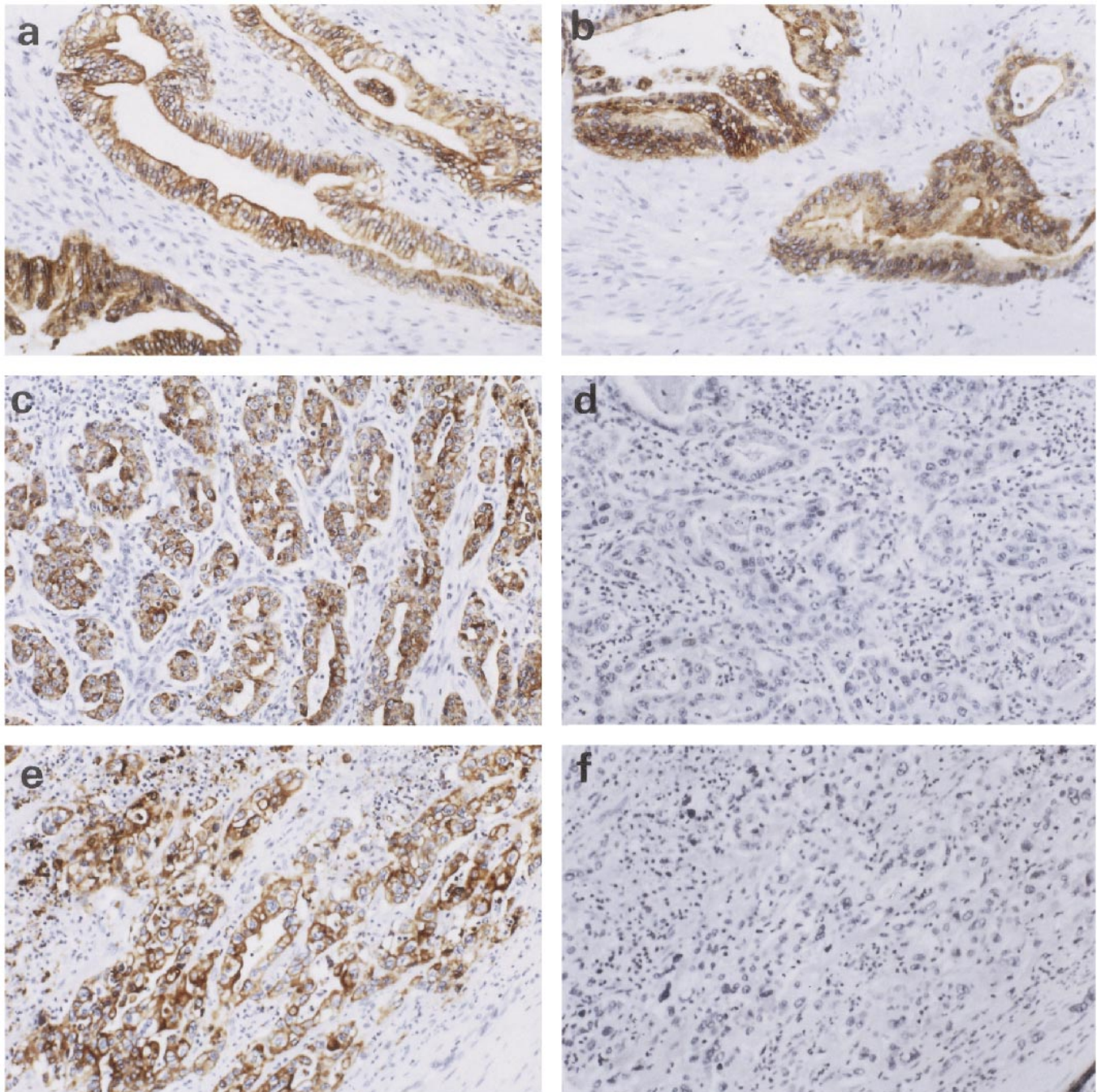
The majority of all tumors were CK7+ and of these tumors, the majority (n = 41) were CK7+/CK20-. The 7 CK7+/CK20+ tumors were from the bile duct, pancreas, or gallbladder. The 5 CK7-/CK20- tumors were equally distributed in the groups. No tumors were CK7-/CK20+. These results are shown graphically in Figure 4.

#### COMMENT

Expression of CK7 and CK20 has been evaluated in numerous carcinomas by several investigators. Cytokeratin 7 has been identified in cholangiocarcinomas of the liver;<sup>1,5,16</sup> in transitional cell carcinomas of the bladder;<sup>1,17</sup> and in carcinomas of the breast,<sup>1</sup> endometrium,<sup>14</sup> ovary,<sup>1,3,17</sup> and lung.<sup>12</sup> Variable expression has been reported in carcinomas of the esophagus,<sup>17</sup> stomach,<sup>1</sup> kidney,<sup>17</sup> and pancreas.<sup>17</sup> Similarly, CK20 has been reported in carcinomas of the colon, mucinous carcinomas of the ovary, transitional cell carcinomas of the bladder, Merkel cell carcinomas, and, frequently, gastric carcinomas.<sup>1-3,15,18</sup> Heterogeneous expression has been reported in cholangiocarcinomas of the liver, carcinomas of the gallbladder and pancreas, and bile duct carcinomas.<sup>1-3,15,18</sup>

Coordinate expression of CK7 and CK20 has recently been proposed as an aid in the determination of the site of origin for a variety of metastatic carcinomas.<sup>13-15</sup> Little is known, however, of the coordinate expression of these cytokeratins for carcinomas of the biliary tract and pancreas. Pancreatic carcinomas have been studied the most, with conflicting results. Some investigators<sup>14</sup> report positive expression for both cytokeratins, whereas others<sup>19</sup> report positive expression for CK7 and negative expression for CK20. Therefore, we sought to expand on the work of these investigators by evaluating the expression of these 2 cytokeratins in carcinomas of the biliary tract region. Our aim was to determine how these carcinomas should be included in the differential diagnoses for metastatic carcinomas when CK7 and CK20 are included in the immunostaining panel. We also questioned whether it was possible, in advanced carcinomas in the porta hepatis, to distinguish gallbladder from bile duct from pancreatic duct origin based on the coordinate expression of CK7 and CK20.

Our results show that most tumors of the pancreas and



**Figure 1.** Immunohistochemical stains showing an adenocarcinoma of the gallbladder with strong staining for both cytokeratin 7 (CK7, a) and cytokeratin 20 (CK20, b); a bile duct carcinoma with strong staining for CK7 (c) and lack of staining for CK20 (d); and a pancreatic ductal carcinoma with strong staining for CK7 (e) and lack of staining for CK20 (f) (original magnification  $\times 20$ ).

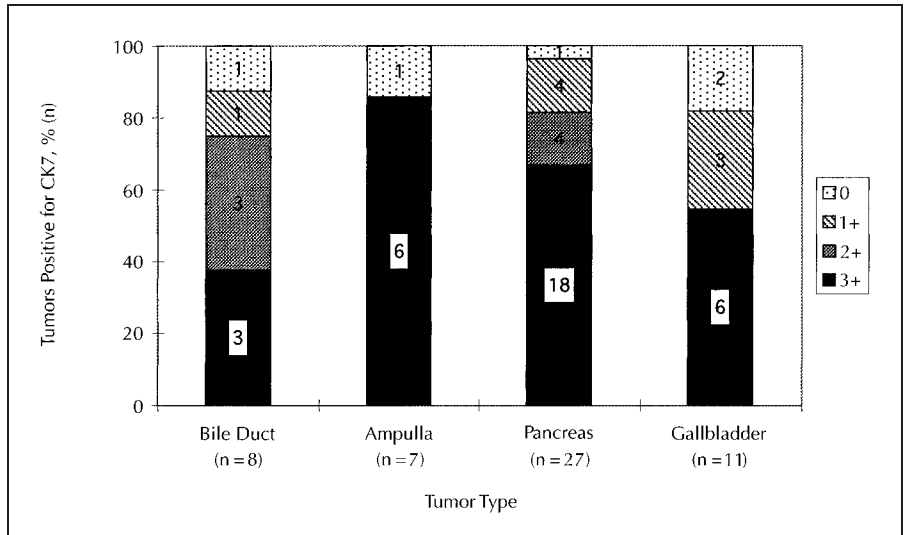
bile ducts, like many other tumors, fall into the category of CK7+/CK20- tumors (Figure 4). This pattern of strong expression of CK7 and negative expression of CK20 was most frequent in carcinomas of the ampulla or pancreas, but the majority of gallbladder and bile duct carcinomas also had this profile. Therefore, it is not possible to differentiate these tumor types based solely on cytokeratin expression. Our results support the previous findings<sup>14,15</sup> that the CK7-/CK20+ phenotype favors gastric and colorectal carcinomas; none of our cases expressed this phenotype.

Interestingly, our results for pancreatic carcinomas, the

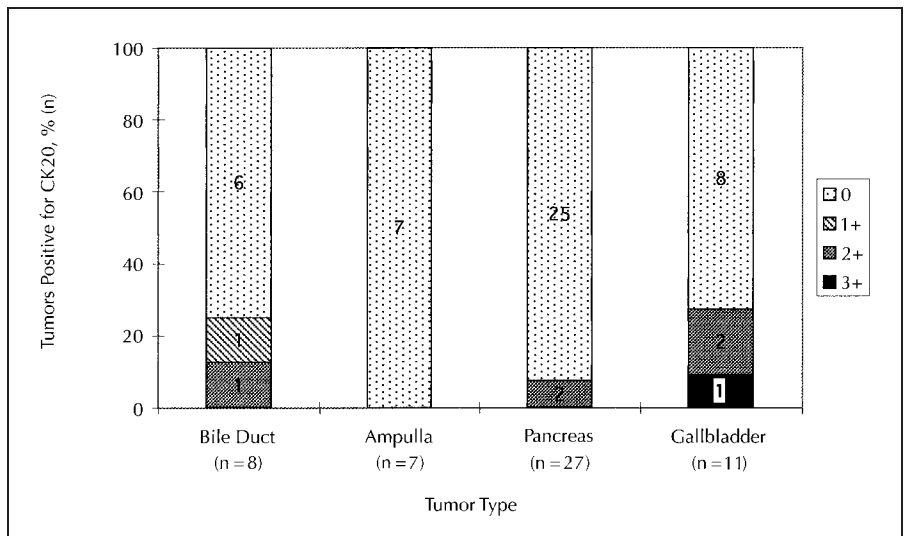
vast majority of which were positive for CK 7 and negative for CK20, did not parallel those of Wang et al,<sup>14</sup> who reported dual expression of these cytokeratins in most pancreatic tumors (26% CK7+/CK20- and 65% CK7+/CK20+). There is no obvious explanation for this apparent discrepancy, except for possible technical differences or the subjective assessment of what constituted positive staining.

An intriguing hypothesis proposed by Wang et al<sup>14</sup> is that CK20 expression differs in mucinous and nonmucinous carcinomas, as reported for ovarian tumors. In ovarian tumors, mucinous tumors express both cytokeratins

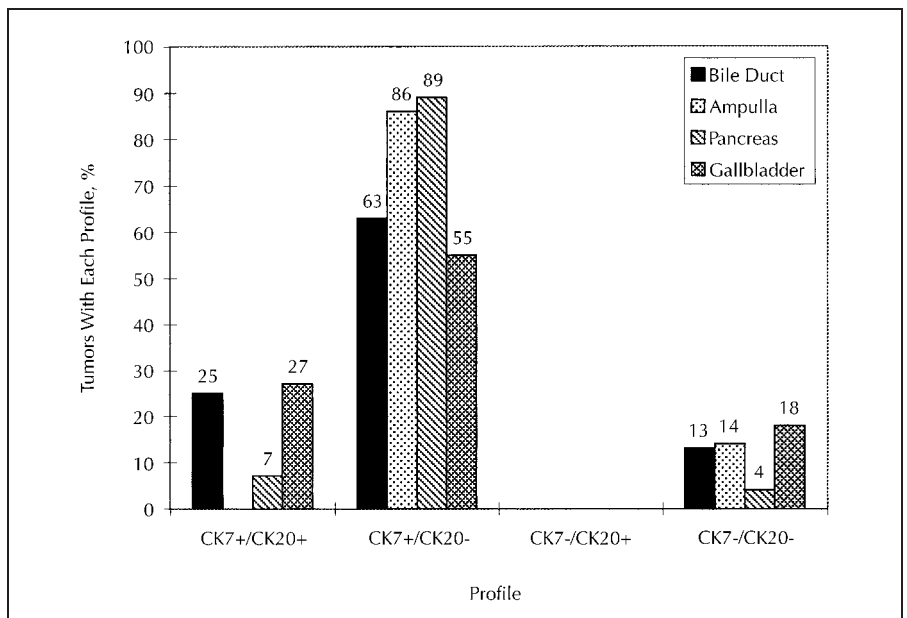
**Figure 2.** Graph showing the percentage and number of tumors positive for cytokeratin 7 (CK7) according to the grade for each tumor type.



**Figure 3.** Graph showing the percentage and number of tumors positive for cytokeratin 20 (CK20) according to the grade for each tumor type.



**Figure 4.** Graph showing the percentage of tumors expressing each cytokeratin 7 (CK7) and cytokeratin 20 (CK20) phenotype for each type of tumor.



(CK7+/CK20+) and nonmucinous tumors express only CK7 (CK7+/CK20-).<sup>14</sup> Dual expression of CK7 and CK20 in pancreatic carcinomas would then be the expected phenotype. Interestingly, even though all of the carcinomas we evaluated were theoretically capable of mucin production, the majority (89%) expressed the phenotype of the nonmucinous (ovarian) carcinomas (CK7+/CK20-) noted in the study by Wang et al.<sup>14</sup> Additional study of cytokeratin expression in pancreatic carcinomas with particular attention to mucinous versus nonmucinous (ie, acinar) subtypes may increase our understanding of CK20 expression.

In summary, our study shows that carcinomas of the pancreas, ampulla of Vater, gallbladder, and bile duct typically express the CK7+/CK20- immunophenotype. These markers should be included in the differential diagnosis of a tumor at a metastatic site as well as the differential diagnosis of other tumors such as non-small cell carcinoma of the lung; bronchioloalveolar, breast, non-mucinous ovarian, and endometrial carcinomas; and malignant mesothelioma.

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